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**INSTRUMENTATION OF AN ISOLATION
CHAMBER FOR THE STUDY OF BRAIN
DEVELOPMENT IN AVIAN MODELS**

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ABSTRACT

The developed work is framed in an international project that aims at the study of sleeping cycles in the developing brain through an avian animal model. It is of great interest to understand the underlying processes of the developing brain in order to address certain conditions of the adult brain that are characterized by sleep-like patterns. The main objective of this Bachelor Thesis is to implement a data acquisition system that involves vital signal processing obtained from avian embryos that would help unlock the unknowns that are involved in the aforementioned sleep-like patterns of certain neurological disorders. The implementation is done according to the design developed by engineers from the Universidad Carlos III de Madrid together with a team of neurobiologists from the University of McGill. The system provides an isolated environment for an avian embryo to develop under controlled conditions while being monitored by a network of sensors that measure EEG, heartbeat and inner and shell temperature. Direct human interaction with the inner chamber is minimized with the aid of a webcam, a microphone, a speaker, a system of lights and a PC interface that allows the user to control and monitor the experiment without interfering with natural development. Future guidelines are proposed, encouraging researchers to make use of the system in order to unlock major unknowns regarding developmental neurobiology and engineers to improve the system with future requirements.

Key words:

Developmental Neurobiology, BCG, EEG, Arduino, LabVIEW

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ACRONYMS

ADC	Analogue to Digital Convertor
BCG	Balistocardiogram
CT	Computed Tomography
ECG	Electrocardiogram
EEG	Electroencephalogram
HFSP	Human Frontier Science Project
IDE	Integrated Developing Environment
IMUs	Inertial Measurement Units
LabVIEW	Laboratory Virtual Instrument Engineering Workbench
MDF	Medium Density Fireboard
NTC	Negative Temperature Coefficient
PET	Positron Emission Tomography
REM	Rapid Eye Movement
WHO	World Health Organization

1 Introduction

1.1 Motivation

This Bachelor Thesis is framed in a bigger research study that aims to identify the moment when an avian embryo's brain starts working for the first time.

It is of great interest to decipher the inner workings of the brain since, unlike other organs, there is little knowledge about its functioning. The unknowns that remain unsolved are preventing researchers from addressing current medical problems that affect a big percentage of the population. In fact, the concern raised by these medical conditions motivated the WHO (World Health Organization) to carry out a screening of neurological diseases in order to put into numbers the amount of people affected by them. With a mathematical model they were able to predict future percentage of deaths caused by neurological diseases [1]. As shown in figure 1, around 12% of the total number of deaths is caused by a neurological disorder, and that number will remain unchanged until, at least, 2030.

Cause category	2005 (%)	2015 (%)	2030 (%)
Epilepsy	0.22	0.21	0.19
Alzheimer and other dementias	0.73	0.81	0.92
Parkinson's disease	0.18	0.20	0.23
Multiple sclerosis	0.03	0.03	0.02
Migraine	0.00	0.00	0.00
Cerebrovascular disease	9.90	10.19	10.63
Polioomyelitis	0.00	0.00	0.00
Tetanus	0.33	0.23	0.13
Meningitis	0.26	0.17	0.10
Japanese encephalitis	0.02	0.01	0.01
Total	11.67	11.84	12.22

Figure 1 - Deaths attributable to neurological disorders as percentage of total deaths. Extracted from [1].

One of the main sources of uncertainty in the field of Neuroscience is the stage of neural development at which the newly-formed neurons cease to act independently and start to behave as a single organ that allows for the complex processes that underlie learning, memory or consciousness. This lack of knowledge arises from the complexity that involves carrying out a neurological research during embryonic development.

Further development of imaging techniques and sensor data acquisition needs to be carried out in order to enable the study of an embryo model. By enabling neurological embryonic development studies a big number of further studies will be unlocked and hence some unknowns related to neurological disorders will be solved. It is of a great motivation to contribute to this process.

The study in which this Bachelor Thesis is framed aims to find and characterize the moment at which the brain awakens and to establish a relationship between brain development and certain latent conditions of the adult organ –such as a coma–. The exploitation of the stimuli that cause activation in the developing brain will enable the development of new therapies to resume normal function in latent adult brains and effectively cure comatose patients. The work performed for the described Bachelor Thesis was carried out in order to enable a study carried out by an international team established at the Hospital General Universitario Gregorio Marañón (HGUGM) in Madrid counting with the contributions of Canadian neurobiologist Evan S. Balaban at McGill University. The project was approved and funded by the Human Science Frontier Program (HSFP), an international program of research support that funds frontier research on the complex mechanisms of living organisms.

1.2 Fundamental Concepts

The fundamental concepts that appear in the context of the research in hand will be presented so as the reader can understand the motivations and conditions that determine the methods followed in the research process.

1.2.1 BCG

BCG (balistocardiography) is a technique used to non-invasively detect the heart-beat of the chicken embryo. Vibration sensors are placed in close contact with the egg-shell in order to detect the small vibrations caused by the beating of the heart. Those vibrations are transmitted through the yolk and the eggwhite to the shell in such a way that the vibration sensors are able to transduce the signal into a measurable electrical

wave. BCG will be further explained in this text, as it is an essential part of the developed system.

1.2.2 PET and CT

PET (Positron Emission Tomography) will be the tool used in order to actively trace radioactively-labelled molecules inside the embryo. PET is a type of nuclear medical diagnosis that provides functional information and would help researches identify the area that is activated in the embryo's brain when exposed to certain stimuli.

A molecule, such as glucose, is radioactively labelled and then injected in the patient or animal model. The labelled molecules travel through the blood stream and different organs within will metabolize them. The radioactive label will decay producing two gamma rays that are emitted in opposite directions. The PET machine detects those rays and renders a functional image that shows the hot spots of cellular uptake [2].

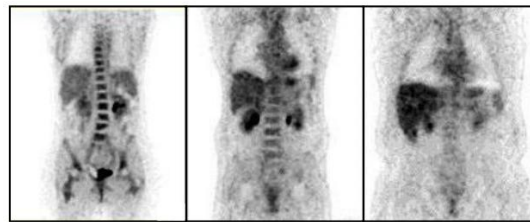


Figure 2 - PET image of a human body. Figure extracted from [3].

In the case at hand a PET-CT scanner is intended to be used. This scanner combines the functional information that PET imaging gives with the anatomical information provided by CT imaging, or in other words, CT imaging anatomically localizes in the body the physiological event represented by PET imaging. CT (Computed Tomography) imaging is a technique that involves x-ray projections reconstructions in order to render a 3D volume of the imaged subject. For small animals, such as a chicken embryo, a higher spatial resolution than in humans is needed. In order to image the chicken embryo a micro PET-CT scanner will be used. This scanner has been developed by a team at the Hospital General Universitario Gregorio Marañón [4].

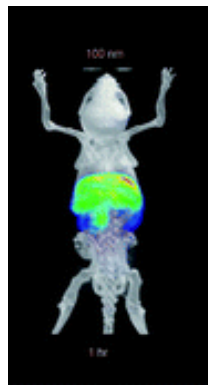


Figure 3 - micro PET-CT image. Image obtained from [4]

1.2.3 EEG

Simultaneously to the use of PET, the EEG (electroencephalogram) signal of the embryo will be constantly monitored. The EEG provides a high temporal resolution signal that represents the electrical activity of the neurons that reach a number of detectors placed on the surface of the head. It is a very useful tool to determine subtle changes in neuronal activity, such as the difference between wake-like brain waves and those associated to dormant states. In the case in hand the brain is much smaller than the human brain, and hence the yield electrical signal will be of smaller amplitude. Therefore, the electrodes will be placed in close contact to the embryo's brain.

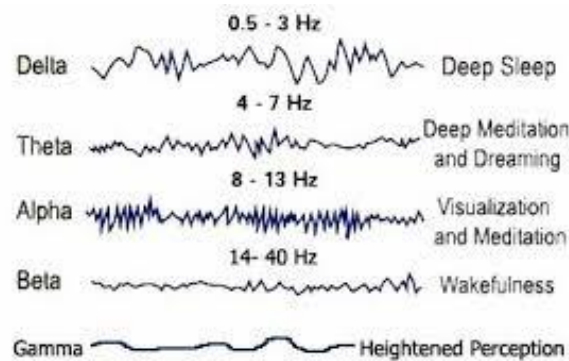


Figure 4 - Different human brain EEG waves.

1.2.4 Embryonic and sleep neurobiology

During fetal development the brain undergoes dormant and active states. In order to detect the stimuli that trigger the change of state an avian model will be used. In spite of the evolutionary divergence, both birds and mammals show a similar pattern of brain activity during sleep [5]. Sleep processes show two different and characteristic patterns that govern the state of the brain and the body. The first pattern is REM (rapid-eye movement), which presents high activity levels in the forebrain and resembles wakefulness. The second pattern is non-REM, and it differs from the former in that it alternates activity in the forebrain with neural quiescence [6]. Since these patterns are caused by the activity of the brain, before its complete development no signal will be detected. Therefore, a combination of radioactively tracing neurological activity and detecting brain signals will be used in order to determine the embryonic stages at which the brain is formed, the brain awakens and complex learning processes start to take place.

1.2.5 Reasons to use an avian model

Avian models are chosen over high vertebrate animals such as small mammals as a matter of experiment optimization. Using oviparous species significantly reduces mortality rates in comparison to small mammals. In addition, the adult mother can be eliminated from the setup of the experiment without causing complications for the development of the embryo. The results obtained for avian models can be extrapolated to human embryos as both share common features regarding the sleeping cycle.

1.2.6 Legal frame

This Bachelor Thesis has been carried out in compliance with the current Spanish legislation [7] that ensures animal safety and comfort as well as safety for the researcher. Future users and developers of the system should act accordingly to the set of directives that were implemented inside the proper legal frame.

All experimental procedures that involve animal manipulation are performed in compliance with the European Communities Council Directive 2010/63/EU and approved by the Institutional Animal Care and Use Committee of the Hospital, supervised by the Comunidad de Madrid according to the Annex X of the RD 53/2013.

1.3 Previous Work

1.3.1 Scientific frame

The research in which this Bachelor Thesis fits is a study carried out by an international team established at HGUGM in Madrid counting with Dr. Balaban (McGill University). This team developed the outlines to carry out an experiment that would determine and identify important processes in the early stages of brain development in avian embryos.

The team decided to proceed by acoustically stimulating the embryo while recording the EEG signal and performing PET imaging studies [8]. The Biomedical Imaging and Instrumentation Group (BIIG) of the Departamento de Bioingeniería e Ingeniería Aeroespacial at the Universidad Carlos III de Madrid together with the Unidad de

Medicina y Cirugía Experimental from the Hospital General Universitario Gregorio Marañón were responsible to establish the experimental procedures in Madrid.

An early design established a system of independent sensors and actuators necessary to carry out the experiment under controlled conditions, as seen in figure 4. [9] The controlled conditions were the temperature and the acoustic and electronic isolation. The only vital signal recorded was the heartbeat. Outside the chamber and with the use of a PET-CT scanner, brain development studies were carried out as explained below.

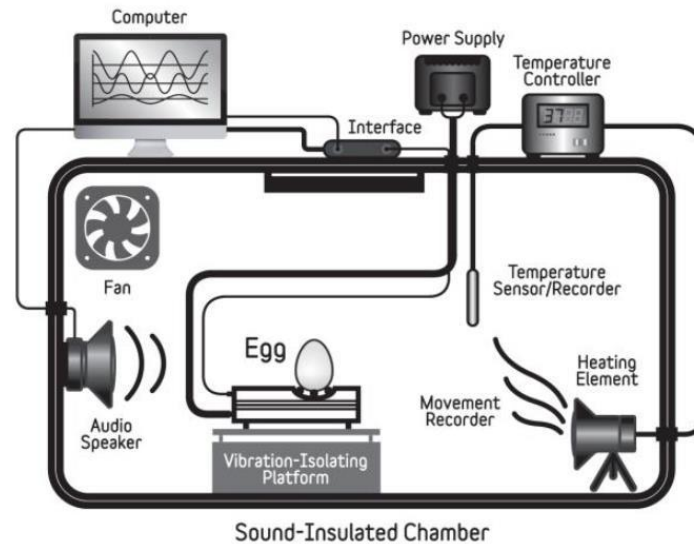


Figure 5 - First system configuration. Figure extracted from [8].

The first experimental procedures were based on the identification of brain states by injecting radioactive tracers, acoustically stimulating the embryo and obtaining PET images after the stimulations. This process is described in figure 6 and illustrated in figure 7.

The embryo developed in an incubator and was put inside the isolation chamber under controlled conditions for experimental purposes. At certain stages of the development a radioactive tracer was injected through a small hole performed in the shell of the egg. It was then put inside the isolation chamber. Once inside, the researchers stimulated the embryo with both authentic and fake mother calls. After some minutes of silence the embryo was anesthetized to avoid movement and a PET-CT scan was acquired. With the obtained image the researchers were able to identify whether some areas of the brain were being stimulated with the real and fake calls. It was very significant to detect the moment when the complex learning processes started and the embryo could differentiate between a fake call and its mother's call.

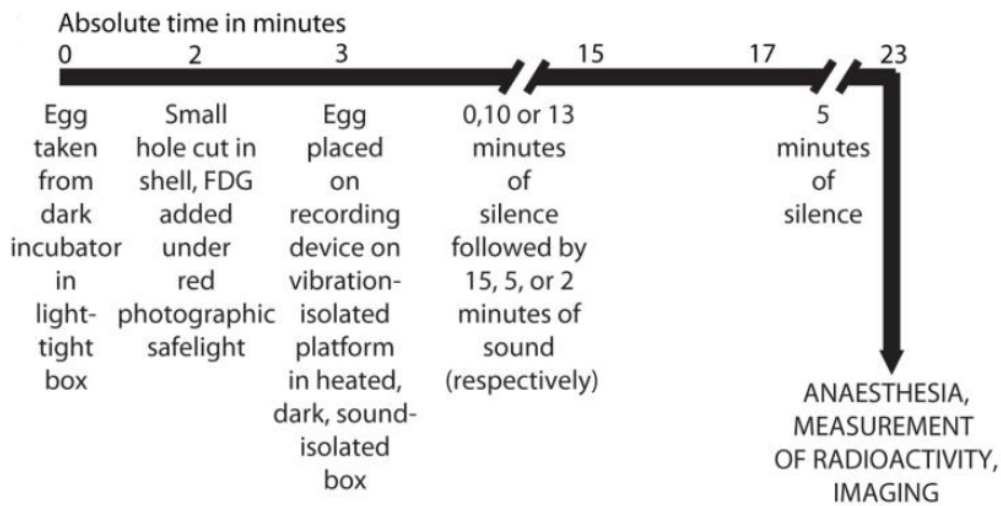


Figure 6 - Schematics of the experiment. Figure extracted from [8].

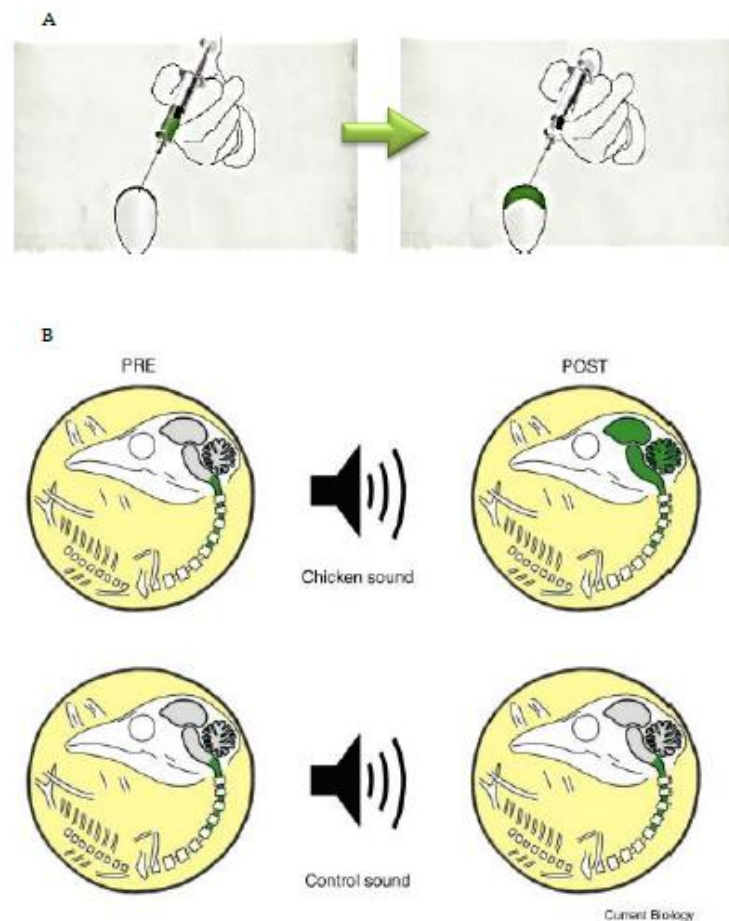


Figure 7 - PET imaging of brain activity. (A) Injection of radiomarker over the breathing membrane through egg shell. (B) Obtained response after meaningful (upper) and meaningless (lower) stimulation. Image obtained from [8].

Through this research project, the conclusion was reached that towards the end of embryonic development wake-like brain function is latently present. Indeed, higher-

brain activity resembling awake, post-hatching animals was observed in all the embryos.

From those results, the team decided to go ahead with the project by implementing new modules to the first design that would allow the researchers to combine more information and reach to new and better conclusions. The first study established the lines to be followed during the second stage of research, described below.

1.3.2 Former Bachelor Thesis work

This Bachelor Thesis is the continuation of the work carried out by biomedical engineer Carmen Fernández Fisac as her Bachelor Thesis [9]. The implementation of the guidelines stated in her work started soon after Carmen presented her thesis. As a result the design and construction of the second generation isolation chamber for the study of embryonic development has been a smooth path conducted by two biomedical engineering students in two consecutive years.

Carmen identified and documented the system specifications and carried out the design of the system according to those specifications. Her work stated as a conclusion that the system was “fully designed and ready for construction”. The following future guidelines were established for future developers to follow:

- 1 Chamber construction
- 2 Integration of individual parts into a sole system
- 3 Development of a user interface
- 4 Design of the automatized system for temperature and humidity regulation
- 5 Evaluation of the system’s final status to move on to animal studies

It was suggested in her work taking advantage of the modular nature of the design so as to make improvements on the components in parallel to the system’s construction. The suggestion was followed by adding a specific goal to this Bachelor Thesis of performing adaptations on the modules that constituted the original system’s design. Moreover, a big importance was given to preserving this modular nature, since it would allow for further improvements and modifications of the different components of the system.

This Bachelor Thesis has been developed in concordance to Ms Fisac outlines, which defined most of the specific objectives.

1.4 Objectives

This Bachelor Thesis fits in a larger, international project entitled “An innovative, interdisciplinary model system for studying the developmental origins of sleep and brain rhythms” which obtained a Human Frontier Science Program (HFSP) research grant in 2013. The team conducting the study is composed of clinical engineers, PhD candidates and physicians as well as the previous participation of Evan S. Balaban (McGill University), Niels Rattenborg, from the Laboratory of Sleep and Flight in Birds at the Max Planck Institute for Ornithology in Germany, and Stefano di Pascoli, from the Università di Pisa. The data acquisition system’s design was initialized by biomedical engineer Carmen Fernández Fisac.

In that context, the **main goal** of the presented Bachelor Thesis is to continue with the started work in order to develop a system that registers vital signs from a chicken *in ovo*, allowing for the study of brain activity of developing embryos in a controlled environment. This second generation system will include automatic monitoring of temperature and humidity in order to ensure a controlled environment under which carry out the experiments. The vital signal monitoring system will include cardiac and neurological signal detection as well as egg temperature. The whole system will be acoustically and electronically isolated. In order to enable the researchers to stimulate the embryo, acoustic inputs and outputs to the system will be included.

The **specific goals** of this Bachelor Thesis are:

- 1 To identify the current situation of the system.
- 2 To plan the steps to follow in order to comply with:
 - 2.1 System specifications
 - 2.2 User specifications
 - 2.3 Technical specifications
- 3 To design the missing components and put together the available ones.
- 4 To develop a user-friendly interface that gathers all information from the chamber.
- 5 To design the automatized system for temperature and humidity control.
- 6 To identify problems and make improvements on individual components.

2 Methods and Materials

The methods to follow and materials to use were first defined with the aid of the User Specifications and Technical specifications. Both documents are attached as appendices and describe the requirements than the system needed to fulfill. A brief summary of the specifications will be introduced for the reader to understand the steps undertaken in the design and development of the final system.

User specifications

The system had to include data acquisition elements that provided the user with information about brain and cardiac activity, temperature, humidity and background noise. The data acquisition system had to be minimally invasive and had to avoid disrupting the functioning of the system. In addition, a constant monitoring of the egg was required while eliminating the window that the first design of the chamber included. The egg monitoring had to be done by both audio and video. Moreover, in order to stimulate the embryo, it was required that the chamber included a speaker.

The user also requested the possibility to control the inner equipment with a console that gathered all the information and control buttons. As far as data acquisition is concerned, it was asked that the data was represented in real-time in the aforementioned console, and that those data were also included in a file compatible with processing tools such as Matlab or MS Excel.

Concerning the conditions for the correct development of the chicken embryo, the system had to be properly isolated both acoustically and electrically. The isolation should ensure that the contact of the inside of the chamber with the outside world is minimal, including minimum human intervention.

Technical specifications

One of the specific objectives of this Bachelor Thesis was to further revise and adjust those specifications. This section presents the previous specifications and a comment on how they have been modified or adapted. Further explanations on the final specifications and results will be presented in following sections of the text.

Monitoring devices should measure EEG signal, heartbeat, embryo temperature, egg temperature, air temperature and humidity, audio and video. Control devices should monitor and vary temperature, humidity, light and audio. Isolation should ensure acoustic, electrical and vibrational isolation. The location of the components should allow easy manipulation and minimal human intervention. Finally, all measured signals should be displayed in a console that should also include the buttons to interact with the inner elements of the system.

Heartbeat signal, egg temperature and audio monitoring devices have not been modified from the specifications proposed in previous work. The heartbeat will be measured with the use of BCG with electromagnetic pickup needles. The egg temperature will be monitored with an industrial pyrometer. The audio monitoring has been adapted to fit any type of microphone with a jack connection.

On the other hand, EEG signal, air temperature and humidity and video monitoring devices have been modified in comparison to those proposed in previous work. The EEG signal communication channel has been adapted to fit either to of the new proposed solution. Further explanations on EEG monitoring are given in following sections of the text. Air temperature and humidity have been chosen to be digital than analog, as proposed. The digital acquisition provides easier communication with the computer for further analysis. Finally, the video monitoring is an infrared webcam instead of a simple webcam with an infrared led ring. These decisions cause no substantial modifications in the purpose of the initial proposals.

As control devices concern, the audio is controlled by the proposed speaker. The lights and heater were not initially built, hence the new components have been chosen in concordance with the system's requirements. The construction of the chamber complies with the isolation technical specifications as the window was eliminated from the design and the walls have been covered with acoustic insulating material.

Finally, the console has been developed in LabVIEW, as suggested in previous work on the system.

2.1 Chamber Construction

The chamber was constructed by the Technical Office at the Universidad Carlos III de Madrid under supervision of the author of this text, following the provided chamber plans. The plans were updated and modified according to specific needs that arose over the time of construction. Both plans are attached as appendices should further information be needed.

The chamber dimensions were determined with values so that the inner components – including the isolation materials– fit inside. The system inside the chamber includes a vibration table –on top of which the BCG and the egg will be accommodated–, a speaker, a microphone, a heating system, temperature and humidity sensors, a pyrometer –which must be located at a proper distance to the egg–, a video camera and a system of lights. Figure 8 shows the final dimensions of the chamber modified from the original dimensions defined in the plans included as an annex.

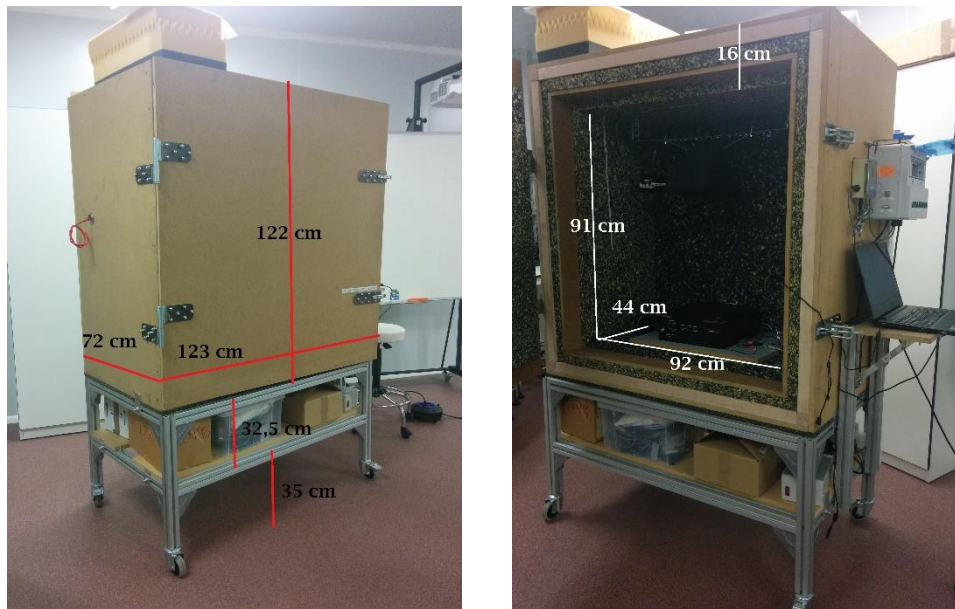


Figure 8 – Final dimensions of the chamber.

A proper environment inside the chamber needs to be created and maintained so as to ensure the embryo's correct development. Such an environment is created and maintained with the sensors and actuators, and enforces with a proper insulation of the incubating chamber. Two types of isolation are required for the correct functioning of the system.

Acoustic isolation is fundamental not only because of the embryo's comfort, but also to ensure minimal interference with the acoustic stimulation carried out during research. This type of isolation was achieved by two means. The first was to design the chamber

in two modules that fit one inside the other. Between the walls of both modules, acoustic insulating material was placed. The second was to minimize the number of open holes to the outside of the chamber. In order to achieve so the door can be hermetically closed and the wire were introduced through two corrugated tubes shaped like an “S” that further reduces noise transmission inside the chamber and trap light form the outside. Once the chamber was finished, it complied with the required 70 dB of noise reduction in the chickens’ audible range, which expands from 0.3 to 10 kHz. Figure 9 shows the schematics on how the materials were finally distributed in the construction of the chamber.

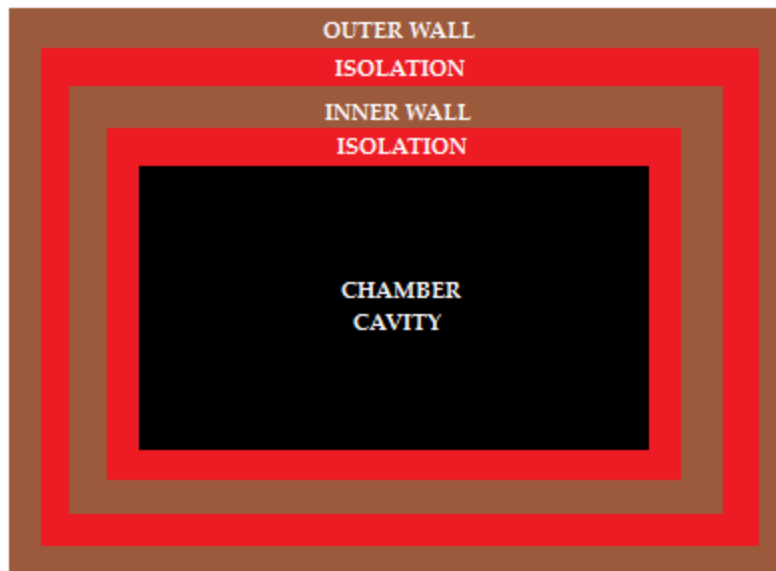


Figure 9 - Schematics of the acoustic isolation of the chamber.

Electric isolation was achieved by avoiding AC-carrying wire in the cavity of the chamber. In order to do the chamber included a lower shelf on top of which several power sources would be placed. On the side of the chamber a control box was added for a correct management of the wires taking current inside the system and bringing information to the outside. Further information on power sources development, control box design and wiring is giving in the following sections, since those were processes that followed the construction of the chamber.

The supporting frame was built with aluminum squared-section beams of 50 mm in order to ensure stability and rigidity of the supporting system. The chamber walls were made of medium-density fiberboard (MDF) because while presenting important characteristics such as resistance and relatively low weight, MDF is easy to manipulate and generally cheaper than natural woods [10]. The characteristics of the insulating material (polyurethane foam panels) were different for the door and the walls of the chamber. For the door a material with higher grain density was used so as to achieve a higher isolation level with less amount of material.

Figure 10 shows some of the steps of the evolution of the chamber's construction process and some of the components of the final design. Figure 10a shows the supporting system for the chamber that was design to both support the heavy weight of the system and provide an easy and comfortable way to transport the chamber by including wheels. Figure 10b shows the outer wall of the chamber. Figure 10c shows the aspect of the chamber and the wall before the second layer of isolation material was added to the design. Figure 10d shows the distribution of material layers in the wall. Figure 10e shows the bolt that ensures the hermetic closing of the door. Finally, figure 10f shows one of the two hinges that hold the door provided its heavy weight and big dimensions.



Figure 10 - (a) Supporting system; (b) Outer walls; (c) First stage of chamber design; (d) Isolation system; (e) Door's bolt; (f) Door's hinge

2.2 Components Identification and Wire Selection

The next step in the development of the incubating system was to identify the components that were available, to determine whether those components were acceptable for the new construction, and to list the missing components in order to proceed to develop or acquire them. Since in further sections the components will be thoroughly described, this section will list the components without detailing their functioning or application.

The available components were the industrial pyrometer, the temperature and humidity sensor, the antivibration table, the EEG, the BCG, the speaker, the microphone, the relays, the analog to digital audio converter and the microcontroller. All of these components showed a proper functioning and only the temperature and humidity sensor and the EEG were chosen to be changed for other similar components.

The missing components were the light, the webcam, the heating system and the computer. Their wiring system was identified before acquisition in order to proceed with the wire selection in order to avoid interruptions in the process.

While the chamber was being constructed, the technic office required the cable bundle so as to introduce it in the S-shaped path through one of the walls of the chamber. Therefore, once the wiring systems of the different components were identified, a list of the needed cables was performed. Then the cable bundle was prepared and implemented in the chamber. Table 1 shows the cables that configured the bundle, their connections and their characteristics.

Cable	Outer terminal	Inner terminal	Color	Quantity	Tag	Comments
RGB 517	BNC RCA – ADC RCA – ADC RCA – ADC +24 V +12 V	BNC BNC Speaker Microphone Heater Light	Black	6	1-6	Impedance: 50Ω
CAROL	±5V Arduino	BNC T&H sensor	Light blue	2	A,B	(R) 22 AωG C3155 60C E60233-8 CL2P (UL)
Brilliance BELDEN	+14 V +24 V	Speaker Pyrometer	(Black) Brown Red	1	-	1509C EI08998-EIW 2PR4 Shielded (UL) CM 2KC
RS-232	D89	Pyrometer	Gray	1	-	α wire H1 P/N 5472C 2PR124 AωG shielded 75C (UL)
USB	USB	Webcam EEG	Gray	2	USB 1 USB 2	Length: 2 m

Table 1 - Cable bundle.

2.3 BCG Design and Development

The cooperative nature of this project made it necessary to build a replica of the BCG used in the first study [8] in order to be sent to Canada to Dr Balaban's team use. The first design was improved in order to include new features such as a better amplification of the signal, a variable notch filter (50Hz for Europe, 60Hz for the USA) and a modular nature of the design that would facilitate further operations on the device. The new BCG plan is attached at the end of the document as an appendix.

The first BCG was built in 2012 for the study of cardiac signals. Three phonographic electromagnetic needles were disposed 120 degrees apart to detect the vibration of the eggshell that, in turn, was caused by the vibrations of the amniotic element. Those vibrations could be caused by the contractions of the embryo's heart, by its breathing movements or by spontaneous movements of the embryo. Figure 11 shows the disposition of the needles and a schematic of the whole circuit.

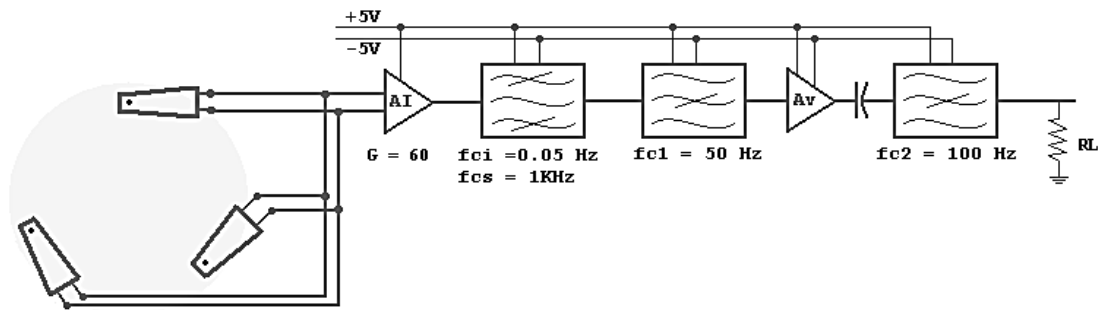


Figure 11 - Circuit of first-generation BCG. Figure extracted from [11].

With the aid of the BCG, researchers in [8] were able to identify different behaviors of the chicken embryo as well as study its interactions with real and fake mother calls, as shown in figure 12.

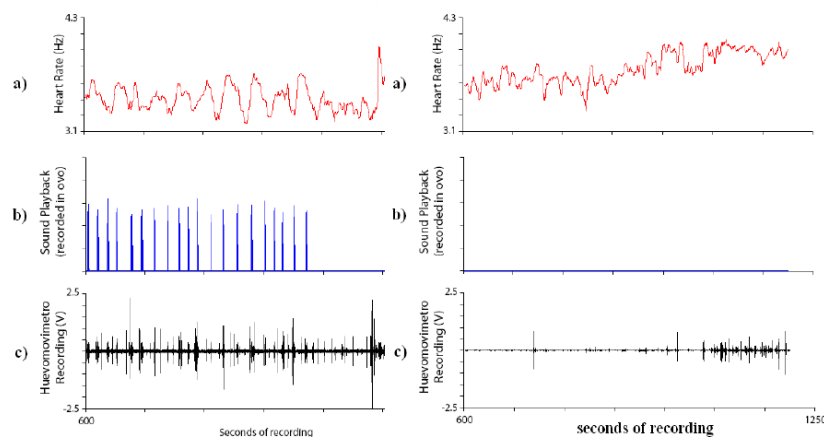


Figure 12 - BCG signal from embryo with and without acoustic stimulation. Figure extracted from [8].

The BCG replica circuit was designed in ORCAD and was printed by the technical office at Universidad Carlos III de Madrid. The components were assembled in the laboratory. Once the new BCG was ready, it was tested and compared against the old BCG, as shown in figure 13. The former version showed perfect functioning while the new one presented technical complications. Hence, the 2012 version has been implemented in the final system while the new version is under trial and improvement.

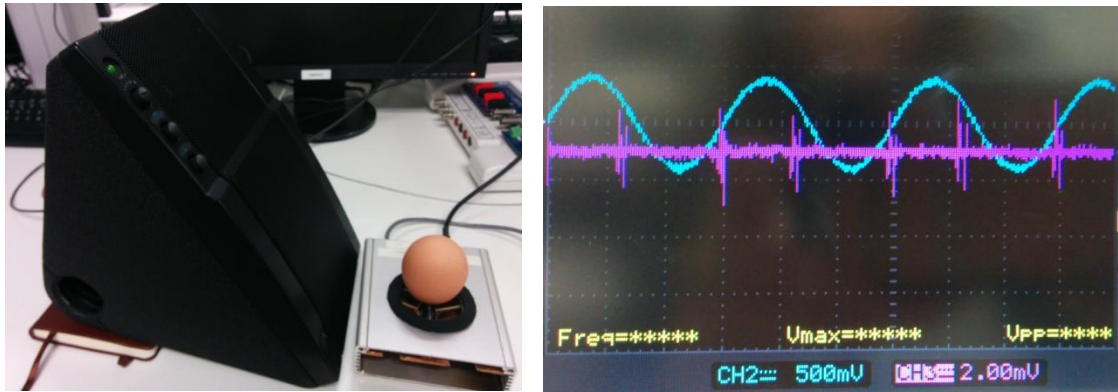


Figure 13 - BCG testing with audio input.

During the development and implementation of the different modules of the system, a great concern was kept regarding compatibility and ease when changing one device for another. In the case of the BCG this was particularly important; not only the connections were not changed, but also the dimensions and functionality of the device were unaltered, as shown in figure 14. This ensures that the moment the new circuit can be exchanged by the old one without further complications.



Figure 14 - New and old versions of the BCG.

The BCG would be wired to a BNC connector, which would allow visualization of the signal with an oscilloscope, and in parallel to an RCA connector that would allow the visualization of the BCG signal in the computer as an audio signal in combination with the signal acquired with the microphone inside the chamber.

2.4 Wiring Process

The wiring process started right after the chamber was built and the components were identified, repaired, acquired or developed. In a first step the cables in the bundle, which was previously introduced through a wall of the chamber, were distributed according to the different requirements for each component, as depicted in figure 15.

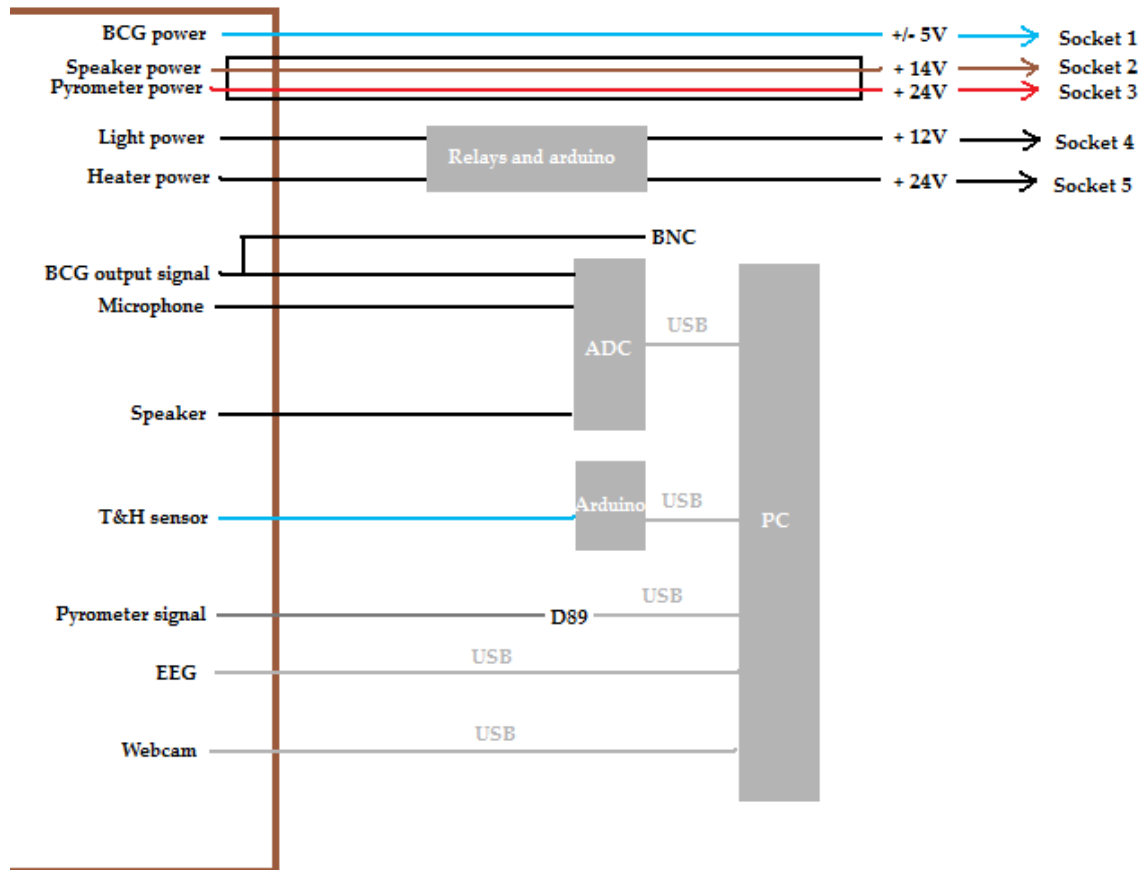


Figure 15- Cable connections. Refer to table 1 for color code information.

Before proceeding to wire the components, the needed power sources were built, as well as the sockets that would connect the power sources with the 220V socket. Moreover, a set of switches was included in the design. These switches would allow the user to turn on and off the alimentation of the different sockets, hence increasing the safety of the system. Figure 16 shows the sockets and the system of switches, as well as the control box where the connections between the inside and the outside of the chamber were made. The control box also hosts the Arduino microcontroller, the audio ADC and the system of relays. This step was fundamental to comply with the user and technical specifications that no AC current would go inside the chamber, that the system allowed minimal human intervention inside the chamber, and that the electric connections were safe for the user.

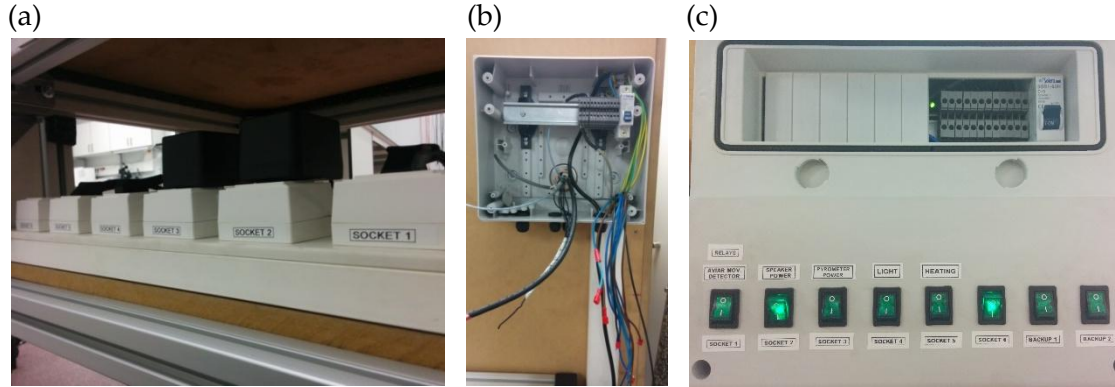


Figure 16 - (a) Sockets; (b) Control box; (c) System of switches.

The speaker, the pyrometer and the light needed no power source development since they were acquired with their own transformers of 220V to 14V, 24V and 12V, respectively. A ± 5 V and a +24 V power sources were respectively built for the BCG and the heater. Figure 17 shows the interior of these power sources.

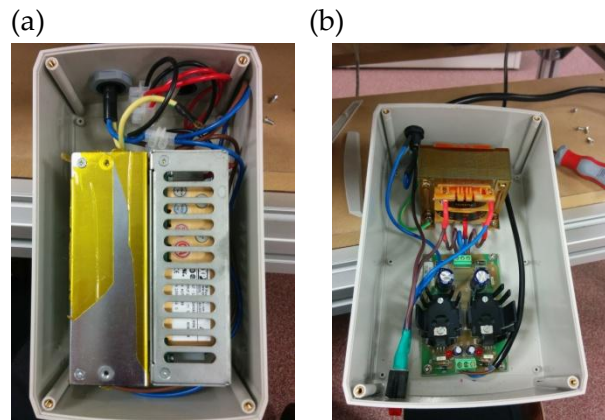


Figure 17 - (a) ± 5 V and (b) +24 V power sources.

As far as the wiring procedure is concerned, the cables were welded to specific connectors to each of their terminations. Following the conditions depicted in figure 15, the following terminals were welded to the cables:

- The cables carrying the BCG, microphone and speaker signals were welded on one side to BNC, jack and RCA connectors, respectively, and on the other side to RCA connectors. These terminals were plugged to an audio ADC convertor.
- The BCG signal was carried in parallel by another cable that was welded to a BNC connector on its outer terminal. This connector was intended to serve as a direct way to visualize the BCG signal with the aid of an oscilloscope.
- The cable connecting the temperature and humidity sensor to the Arduino microcontroller was welded on both sides to simple pins.

- The RS-232 cable was welded on one side to the pyrometer's RJ11 connector and on the other side to a DB9 terminal, following the code shown in figure 18.

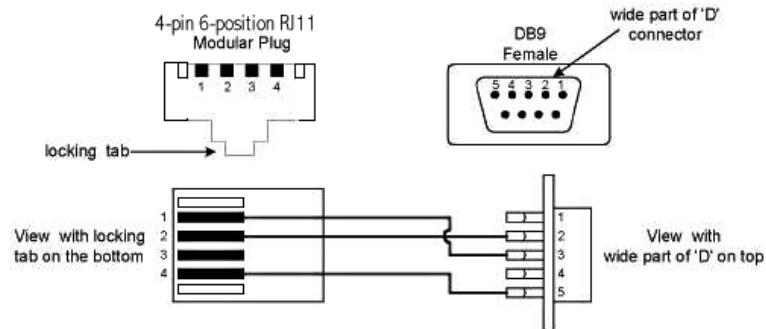


Figure 18 - RJ11 to DB9 serial port correspondence. Figure extracted from [12].

- Both USB cables have been left unaltered. USB-1 is directly connected to the webcam through USB connection. USB-2 would be directly connected to Stefano di Pascoli's EEG sensor or opened and adapted to Nathalia Peixoto's -associate professor of the Electrical and Computer Engineering Department at George Mason University- EEG sensor. Further information on the selection of the EEG sensor is detailed in following sections.
- Cables carrying power were welded on one side to the proper terminal that would ensure proper connection to the component. On the other side they were connected to the terminal box that in turn connected them to the cables plugged to the sockets.
- Two of those cables (the ones that brought the power to the lights and the heater) had the positive wire connected through a relay to the terminal box and the ground wire directly connected to the terminal box. The system of relays was in turn controlled by the Arduino, as explained in further sections of the text.

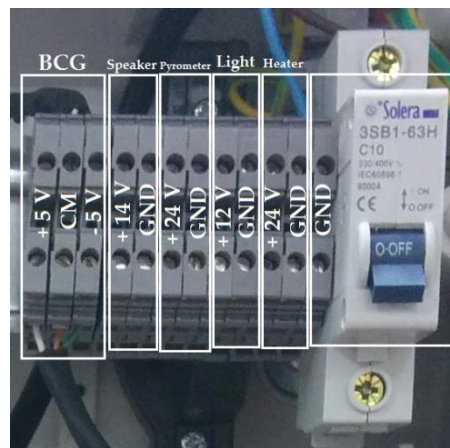


Figure 19 - Terminal box for power connections.

2.5 Software Design

Software design was carried out with the intention of creating a single console with which the user could manipulate the actuators as well as visualize the data acquired by the sensors inside the chamber. For this purpose, the connections of the components to the computer were established as depicted in figure 15. The lights and the heater would be turned on and off according to the instructions given by the Arduino microcontroller through a system of relays. The temperature and humidity sensor would send the information through Arduino communication. The speaker, the BCG and the microphone would be connected to the computer through an audio ADC. The webcam, the pyrometer and the EEG would be connected to the computer through USB communication.

LabVIEW

The console was designed in the LabVIEW programming platform. LabVIEW (Laboratory Virtual Instrument Engineering Workbench) is a visual programming environment created by National Instruments (NI) that uses a high-level dataflow graphical language in which execution is dictated by the block-diagram structure [13].

The programmed console would allow the user to interact with the inner environment of the chamber by switching on and off the lights, visualizing temperature and humidity information and playing and acquiring sounds.

Data from the sensors as well as commands to turn on and off the lights are retrieved from and send to the Arduino microcontroller through serial communication.

Arduino

The Arduino software is an open-source IDE (Integrated Development Environment) that provides an intuitive way to write the code that controls the microcontroller's board [14]. The environment is written in Java and is based on open-source software. Arduino software is uploaded to the board through a USB connection.

The code written in Arduino established the serial communication with the LabVIEW platform. Arduino would read the instructions given by LabVIEW and would send the data from the sensors to be displayed on the console.

Without serial communication with LabVIEW, the Arduino code controlled the temperature and humidity levels inside the chamber by opening and closing the relays that controlled the power supply to the heating system.

3 Results

This chapter presents the result of the work performed following the steps described in *Methods and Materials*. In this chapter the different components of the system are thoroughly described and a calculation of the total budget spent in the project is presented. The system has been classified in sections that correspond to different user specifications such as the chamber, the monitoring of vital signs, the control of air temperature and humidity, the system for acoustic stimulation of the embryo, the system of lights and video recording and the user-friendly interface. The general distribution of the system's module is presented in figure 20.



Figure 20 - Final state of the system.

3.1 System components

In order to fully understand the final state of the system and the purpose of the followed work, the result is presented following sections corresponding to the functionality of each group of components. Each of those groups was developed with the intention of solving different user and technical requirements.

3.1.1 Chamber and isolation

Chamber

The chamber has inner dimensions 92x44x91 cm that ensure enough capacity to accommodate the modules that integrate the different system's components. These dimensions also allow the system to have enough oxygen capacity for the egg without the need of ventilation, which would highly increase the acoustic noise in the inner cavity. The inner cavity hosts a volume of air of 368 l, while chicken oxygen consumption is 183, 558, 638, 1003 and 1338 ml/day for 12, 16, 18 day old embryos, externally pipped eggs and hatchlings, respectively [15]. Since the egg will not stay for longer than a day inside the chamber, the volume of air that the inner cavity hosts is more than enough for normalized oxygen consumption of the developing embryo. The outer appearance of the chamber is depicted in figure 21.



Figure 21 – Outer appearance of the chamber.

The chamber can be divided in three sections for illustrative purposes that allow an easier understanding of the system's final design. The first section is the lower part, which shelters the sockets and the power sources. The second section is the lateral side through which the cables were introduced. The third section is the cavity of the chamber, where the different components were installed.

The cavity, as shown in figure 22a, sheltered the sensors and actuators of the system. Its size was limited by the number of layers installed for acoustic isolation purposes.

The lateral part, as shown in figure 22b, hosted the cables installed through the chamber's wall, the PC and the control box. The control box in turn hosted the relays, the Arduino microcontroller, the audio ADC and the connections of the chamber cables with those connected to the sockets.

In the lower part, as shown in figure 22c, six sockets were installed and five power sources were placed ($\pm 5V$, $+14V$, $+24V$, $+12V$ and $+24V$).

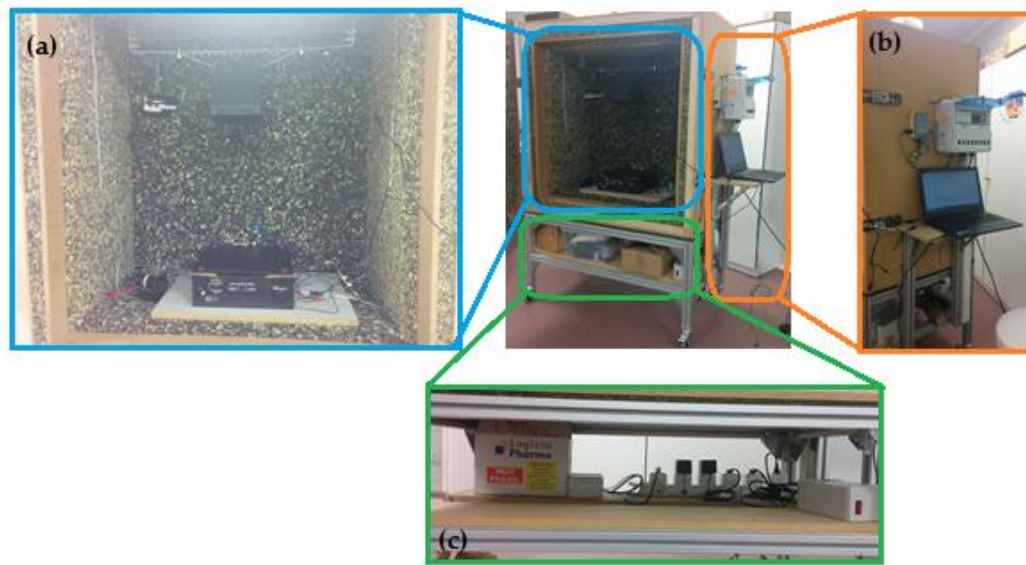


Figure 22 - Sections of the chamber: (a) inner cavity; (b) lateral side and (c) lower part.

Electromagnetic isolation

As far as electromagnetic isolation is concerned, required to minimize the effect of the 50/60Hz noise, no 220V power was introduced inside the chamber's cavity, for which purpose the lower part of the design was built to host the low-voltage power sources.

Acoustic isolation

Proper development of the chicken embryo required certain conditions including a limited range of audio waves' amplitude and frequency. Provided that the developing embryos' are half deaf due to muscle relaxation and fluid damping of the middle ear and that the chicken's audible frequencies range from 30 Hz to 10 kHz [16], an attenuation of 70 dB in that range was required. The soundproof environment was achieved by alternating layers of MDF with layers of polyurethane foam panels, as shown in figure 23a.

Moreover, an S-shaped path of corrugated tube was designed for the power and signal cables to be introduced in the cavity of the chamber, as shown in figure 23b. The path was included inside the right-most lateral wall of the chamber and an extra path was implemented on the opposite side in case more cables were needed during the wiring process. The S shape would trap the noise of the outside by damping the waves in the turns of the path.

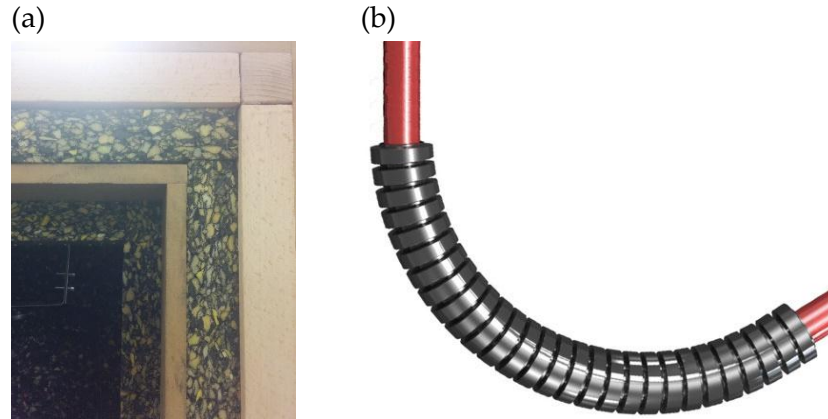


Figure 23 - (a) Layers of acoustic isolation and (b) s-shaped cable bundle path of corrugated tube.

Isolation from vibrating motion

There is a technical requirement of isolating the gentle vibrations produced by the embryo's heartbeat, breathing motion and spontaneous movements from the vibrations of the outside environment. For this purpose, an antivibration table was included in the final setup of the system on top of which the BCG was placed.

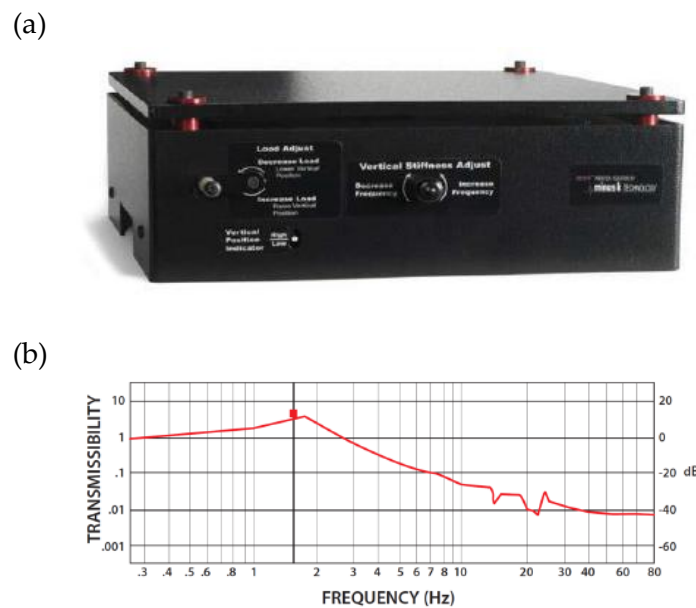


Figure 24 - (a) Antivibration table and (b) its behavior. Figures extracted from [17].

The selected table was the BM-10 Bench Top Vibration Isolation Platform, shown in figure 24a. This device can bear up to 4.5 Kg, which is by far sufficient for the BCG and egg's weight [17]. The device achieved better performance than air tables and provided and easier manipulation procedure. The table was placed inside the chamber's cavity, and on top of it the BCG device was placed. After doing so, the red collars were carefully removed and the table was ready to perform [17] according to the behavior shown in figure 24b.

3.1.2 Vital signs monitoring

The user specifications required that the system included EEG, cardiac activity and temperature of the embryo acquisition and recording.

Electroencephalogram (EEG) acquisition system

There are two systems that have been contemplated as possibilities for EEG recording. Both systems were taken into account in the wiring process and a USB cable was left unused allowing for easy connection of either EEG recording device. The characteristics and specifications of each system are presented, in order to allow future users to select the one that suits best for the requirements of the experimental procedure.

The first system was designed and developed by an Italian team lead by Stefano di Pascoli at the Università di Pisa [18]. The device has a transmitter that communicates with the receiver through a radiofrequency link. The transmitter, shown in figure 25a, acquires the signal from the electrodes and eliminates the DC component. The transmitter has small size and weight and low power consumption, and can be turned on and off with the aid of a magnet. The signal is fed to a microcontroller via a 6-channel 16-bit ADC. The microcontroller encodes the data for radio transmission. This data is received by the antenna shown in figure 25b, which, in turns, sends the signal to the integrated system shown in figure 25c. This circuit amplifies, demodulates, and reformats the data to be transferred via USB. Figure 25d shows the receiver and its USB connection.

Figure 25e depicts the connections of the system to the computer and figure 25f shows how the electrodes are connected to the transmitter and how they are implanted in the embryo's brain through an opening in the shell. A user-friendly software has been developed for the device. Once it is installed and the USB is connected to the computer, the data will be automatically displayed on the screen, as shown in figure 25g. If future researchers wish to implement this EEG data acquisition system, they would simply need to connect the USB wire to the one that is already installed in the chamber, and then connect the outer end to the computer.

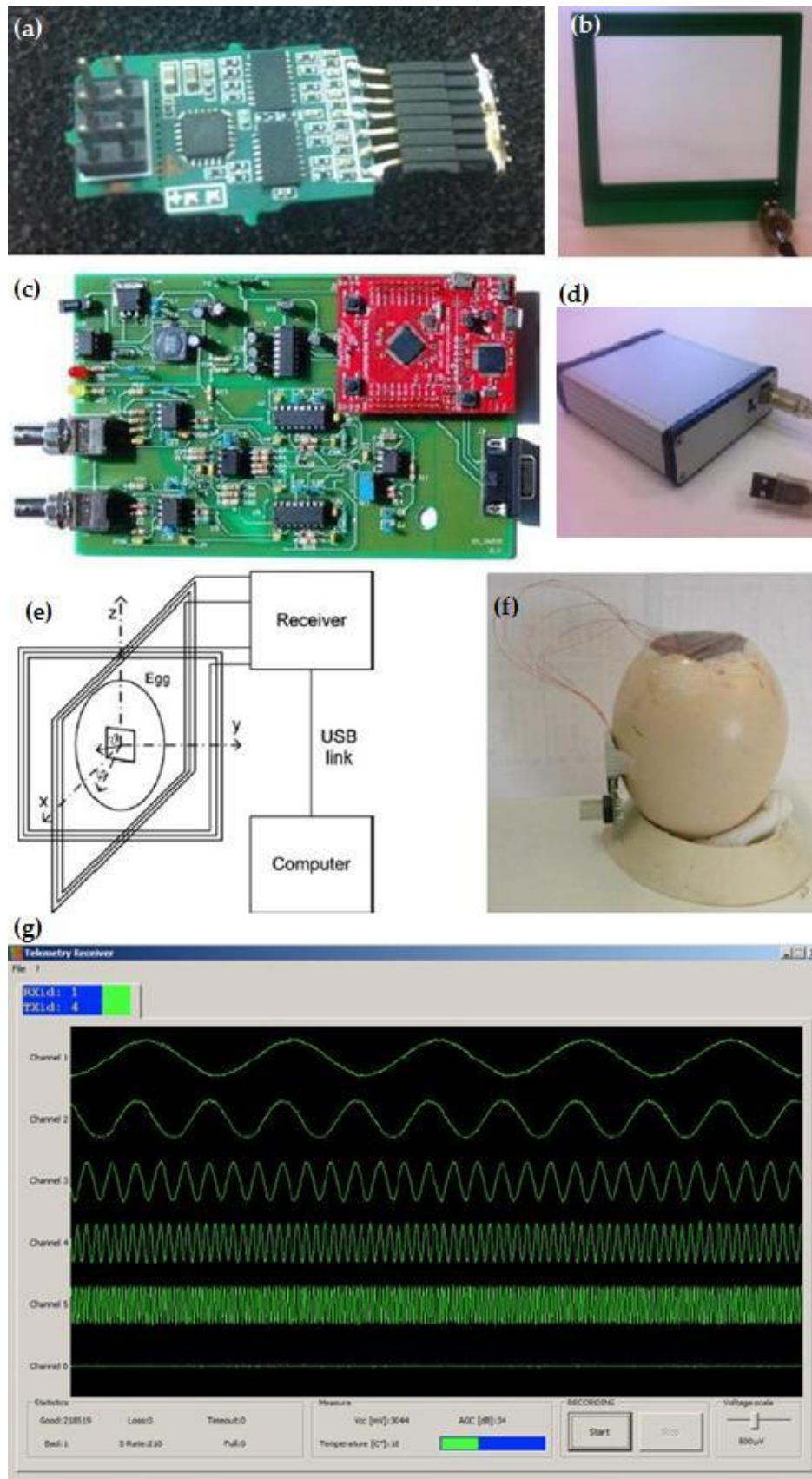


Figure 25 - EEG system developed by Stefano di Pascoli at Università di Pisa. (a) Transmitter; (b) Antenna; (c) Receiver's integrated circuit; (d) Receiver and USB connection; (e) Schematic of the overall system; (f) Real implementation of the system; (g) Display on the computer.

The second system was designed and developed by Nathalia Peixoto, and it is a very small and flexible component that includes six pads. Figure 26a shows the electrode, which is 4cm long. The left part of the thin layer of Kapton that includes the electrodes is placed in direct contact with the cortex. The right-most part is connected to long wires that are in turn connected to an amplifier. The chosen one, shown in figure 26b, is the High Performance Grass amplifier [19]. This model has high input impedance (20M Ω), featuring amplification values up to 200.000, a low noise, a built-in calibrator and high and low pass filters. A low-noise 24V power source, shown in figure 26c, was built for this amplifier. Data is visualized on the computer with complementary software (PolyVIEW™) that acquires, stores and analyzes the amplifier's output. Figure 26d illustrates the visualization on the computer.

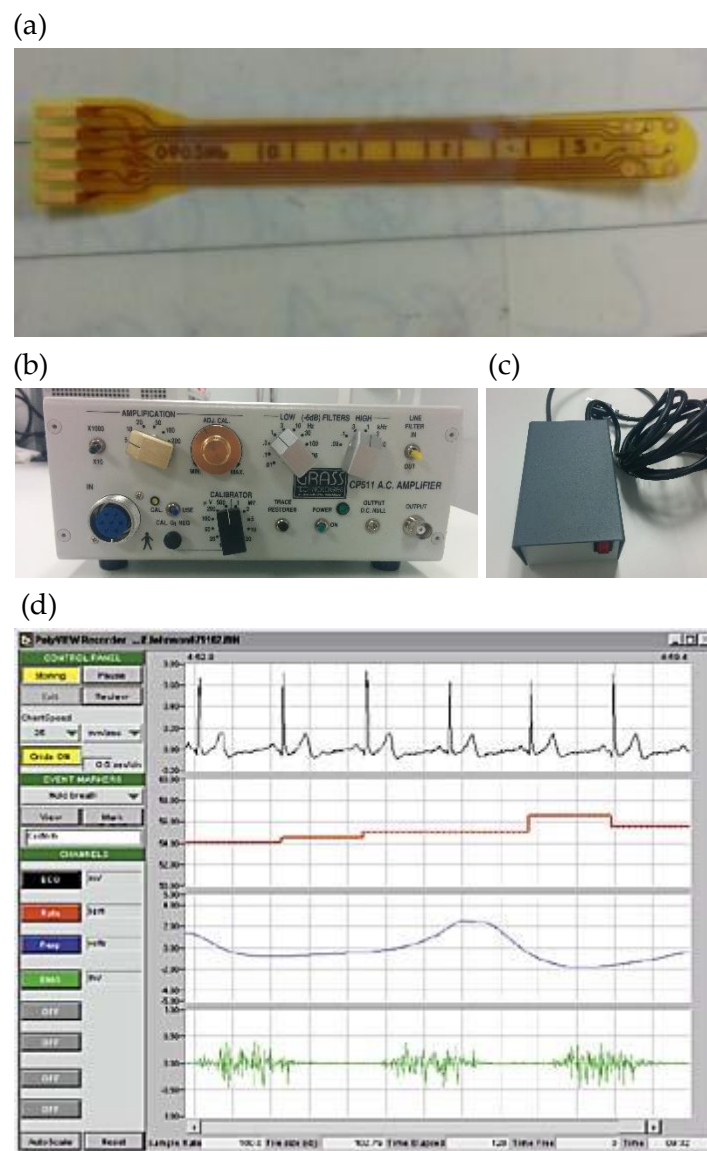


Figure 26 - (a) Electrodes; (b) GRASS amplifier; (c) 24V Power source; (d) PolyVIEW.

Cardiac activity acquisition system

For cardiac activity measurement purposes a balistocardiogram (BCG) was developed and installed. As mentioned in previous sections, a BCG provides a non-invasive way to measure the embryo's cardiac activity. It is extremely difficult to measure such a signal with a convention electrocardiogram (ECG) due to its low amplitude; hence the use of the BCG seemed more appropriate to yield accurate signals.

In order to detect the gentle vibrations caused by the heartbeat of the embryo as well as its spontaneous movements, phonograph needles were used. The needles that were used in the new design are electromagnetic phonograph needles, shown in figure 27. This type of needles picks up the vibration thanks to the movement of the tip, which is suspended by a cantilever coupled to a coil immersed in a magnetic field. These movements of the coil in the magnetic field induce a small electric current that is amplified and digitized in the following stages.



Figure 27 - Phonograph needle.

Three phonograph needles were placed 120 degrees apart as explained in the chapter *Methods and Materials*. The BCG was placed on top of the antivibration table and the egg was thought to be located on top of the phonographic needles, as shown in figure 28. The BCG required a power supply of $\pm 5V$, which was provided by a power source built for the cause. The output signal of the BCG was connected in parallel to two coaxial cables to the outside of the chamber. One of the cables was connected with an RCA terminal to an audio ADC. The other cable could be connected to an oscilloscope with the aid of a BNC connector.



Figure 28 - Positioning of the egg on the BCG.

As mentioned in previous sections of the text, a second-generation BCG was designed and developed. The new design compared to the old one included some improvements while maintaining quality of performance. Those improvements were mainly intending to provide a more intuitive distribution of the circuit elements, as well as an easy way for researchers to make further improvements in the board. In order to achieve so, the phonograph needles were separated from the main circuit in a two-level design of the device. Moreover, two notch filters were included; one for use in North America (60 Hz noise removal) and the other for use in Spain (50 Hz noise elimination). The appropriate filter can be easily selected with the aid of a switch located on the outside of the box hosting the device. The phonograph needles were connected in a way that enables future researchers and developers to replace them by new phonograph needles or even by other systems of vibration detection such as optical methods. Figure 29 shows the final state of the new BCG device.

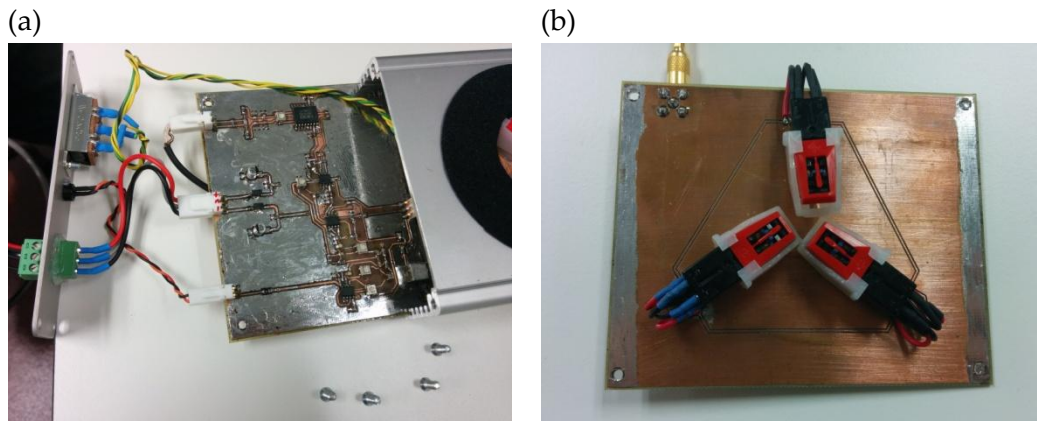


Figure 29 – (a) Circuitry of the second-generation BCG; (b) Phonographic needles.

The system was designed so as to enable easy connection of either the old or new models.

Embryo's temperature acquisition system

The temperature of the embryo will be related to the temperature of the egg's shell, which will be measured with the aid of a pyrometer. The one installed in the chamber is the OS552 Industrial Infrared Thermometer by Omega [20]. Pyrometers provide a way to non-invasively measure a body's temperature. The principle of operation is to detect the blackbody radiation emitted by a particular object [21], which is the electromagnetic radiation emitted by an object in thermodynamic equilibrium [22]. The radiation can be related to the temperature of the object through the Stefan-Boltzmann law (equation 1, [23][24]).

$$j^* = \varepsilon \sigma T^4 \quad (1)$$

Where j^* is the thermal radiation of the object (detected by the pyrometer), ε is emissivity of the object, σ is the constant of proportionality and T is the temperature at which the object is in equilibrium with its surroundings. The emissivity of the egg's shell was calculated to be 1.00 after calibration with a PanLab HB 101/2 probe and thermometer [9].

The installed pyrometer has set emissivity value 1.00 as default, so no further calibrations were needed. Moreover, the infrared thermometer works in a temperature range from -18°C to 538°C , which suits with the requirements of the experiment in hand. The accuracy of the pyrometer is $\pm 1\%$ of reading and it has a fast 250 ms response time. The thermometer has two parts, the detector and the circuitry, as shown in figure 30a. Both parts were installed in the cavity of the chamber, and the output was connected through RS-232 serial communication to the PC. As far as physical location is concerned, the pyrometer was located at a distance such that the field of view of the pyrometer fully falls on the egg's shell, as shown in figure 30b.



Figure 30 – (a) Pyrometer's circuitry and detector and (b) condition for physical setup.

The pyrometer is powered with a +24 V power source that was included in the acquisition of the device. As for all other components, the power source was located outside the chamber in order to avoid AC current inside the cavity.

The information provided by the pyrometer was not implemented in any automatizing process, as it would be displayed solely for informative purposes.

3.1.3 Air temperature and humidity

Air temperature and humidity measurements were required to be not only recorded and displayed but also automatically controlled and maintained at a fixed range by the system. There are three components that configure the air temperature and humidity system. First of all, a temperature and humidity sensor was installed inside the chamber. Secondly, a heater was installed in order to raise the temperature when it

dropped below a certain value. And finally, with the aid of the PC, the Arduino microcontroller and a relay, the heating system was automatized.

Temperature and humidity sensor

The chosen air and humidity sensor (figure 31) was the Grove Temperature and Humidity Sensor, provided by Seeed [25]. It is a modular sensor that was designed with the purpose of being compatible with the Arduino microcontroller and its programming language. The sensor provides a pre-calibrated digital output with the temperature and humidity information. Relative humidity is measured with a capacitive sensor element and temperature is calculated with the aid of a negative temperature coefficient (NTC) thermistor. The sensor works in a range of 20% - 90% RH and 0 ~ 50 °C, being the required ranges inside the provided ones. The accuracy of the measurements is $\pm 5\%$ RH for humidity and $\pm 2^{\circ}\text{C}$ for temperature, with signal collecting periods of 2 seconds and a sensitivity of $\pm 1\%$ RH and 1°C .

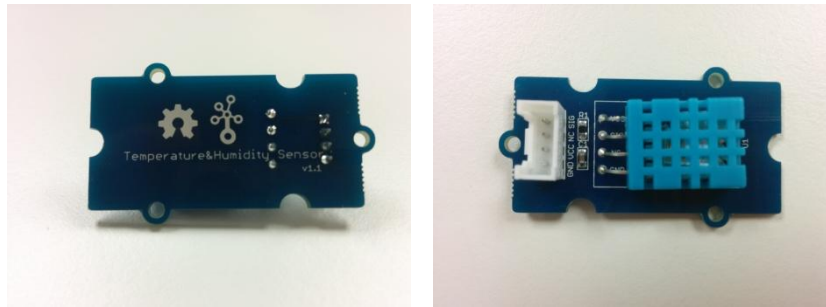


Figure 31 - Grove Temperature and Humidity Sensor by Seeed.

The sensor was connected to the Arduino microcontroller through three wires that provided a power of +5V to the sensor and that carried the signal output to the Arduino. The output signal was connected to the analog input A0. In spite of being a digital signal, the Arduino library for the sensor manipulation has been optimized to work through the analog pin [26]. A schematic of the connection is shown below.

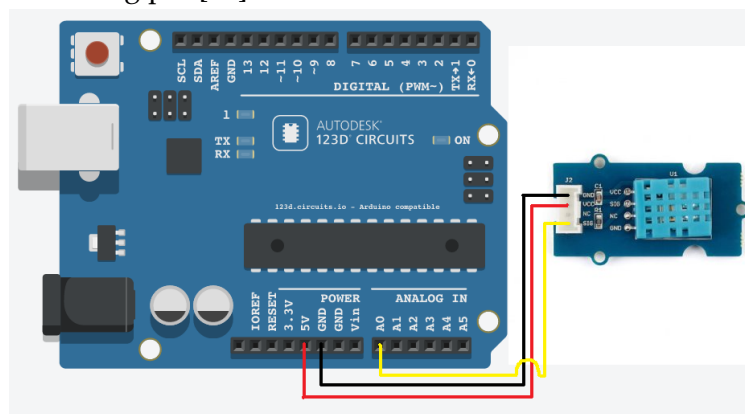


Figure 32 - Connections of the sensor to the Arduino Uno board.

Heating system

A heating system was designed and developed according to the necessities and requirements of the project in hand. Four power resistors (figure 33a) were mounted on a metal radiator that was then attached to a flexible support, as shown in figure 33b.

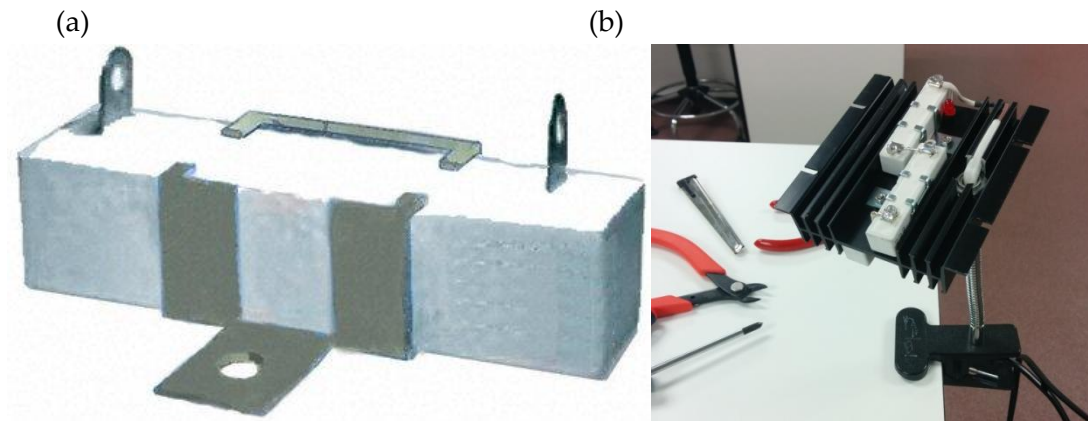


Figure 33 - (a) Power resistor; (b) Mounted heater.

The resistors were provided by Tyco Electronics. The resistive elements are deposited in a ceramic core that is embodied in a ceramic case [27]. They provide efficient thermal performance at a low cost, while allowing for designs such as the one envisioned for this project. The heater is powered with a +24V source built for the purpose. The resistors –with value of $3.3\Omega \pm 5\%$ [27]– as shown in figure 34.

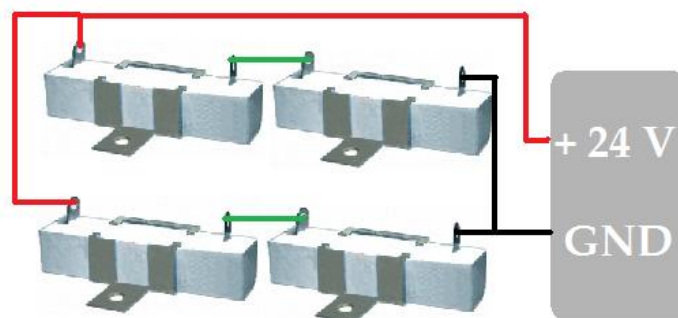


Figure 34 - Wiring of the resistors to the power source (schematics for illustrative purposes).

In order to control the humidity of the air, a water container was placed in close proximity to the heater. The amount of water was slowly increased until the desired humidity response was achieved.

Automatized temperature and humidity regulation

The temperature and humidity values are solely controlled by the Arduino board. The code behind this performance is attached as an appendix at the end of this text. The Grove sensor has been designed to perform in an easy way when connected to Arduino by making use of the Grove library. This library includes functions that retrieve temperature and humidity data. The code iteratively calls those functions and checks whether the temperature and humidity values are within the stipulated ranges of 25-38°C for temperature and 20-30 %RH for humidity. If the temperature drops below the lower threshold a digital output pin is set HIGH. This pin is connected to a relay that opens and closes the power supply of the heater. When the pin is set HIGH the relay is closed and the heater is therefore turned on. Figure 35 shows the schematics behind the described process.

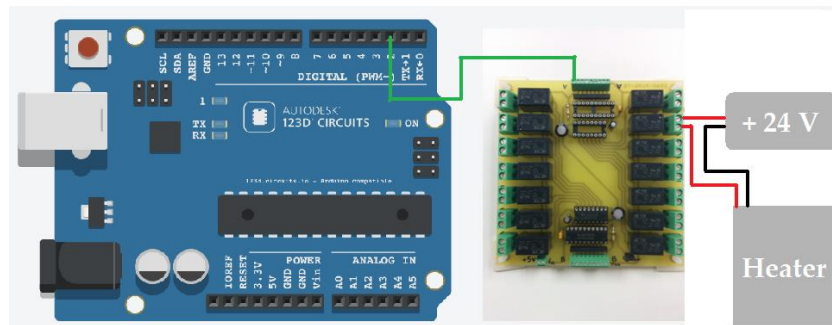


Figure 35 - Schematics of the connections between the board, the relay, the heater and the power source.

In order to determine the relationship between the amount of time the heater was on and the time it took for the temperature to rise a certain amount, several trials were made while saving the measurements in a log. The heating system was then manually calibrated. Figure 36 shows the final relationship of the time the heater was on to the time it took to increase the temperature of the system. Taking into account the trials, it was decided that the heater would be automatically turned on every time the temperature was below 25°C and off every time the temperature reached 28°C. Hysteresis prevented complications with the system of relays.

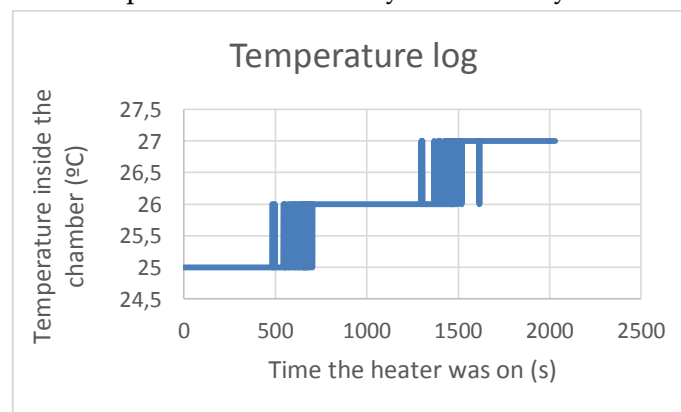


Figure 36 - Temperature log. Since the thermometer has a precision of $\pm 1^\circ\text{C}$, the transition between two consecutive degrees appears to oscillate.

Display of temperature and humidity values

For the user to know the real-time temperature and humidity values Arduino was connected to LabVIEW through serial communication for display purposes. Information on how the connection was made, as well as how the data were displayed, is given in the *User interface* section of the chapter *Results*.

3.1.4 Acoustic stimulation

Regarding the core of the research study, the user specifications required the instalment of a speaker to play sounds to the developing embryo as well as a microphone to record the played call and other sounds inside the chamber.

Speaker

The researcher team requested the instalment of a device that would allow them to stimulate the chicken embryo with two different audio files: the mother call and the fake sound. The speaker was required to create at least a 90 dB pressure to reach and affect the embryo. An Advent AV570 speaker by Audiovox [28] was acquired, as it complied with the technical specifications by providing a 70 W power when connected to a 14 Vdc and 0.8 A power source [9]. The speaker is shown in figure 37a and the power source in figure 37b. The loudspeaker was mounted on a metallic bar that was secured to the top wall of the chamber, in such a way that the device would hang over the egg, as shown in figure 37c.

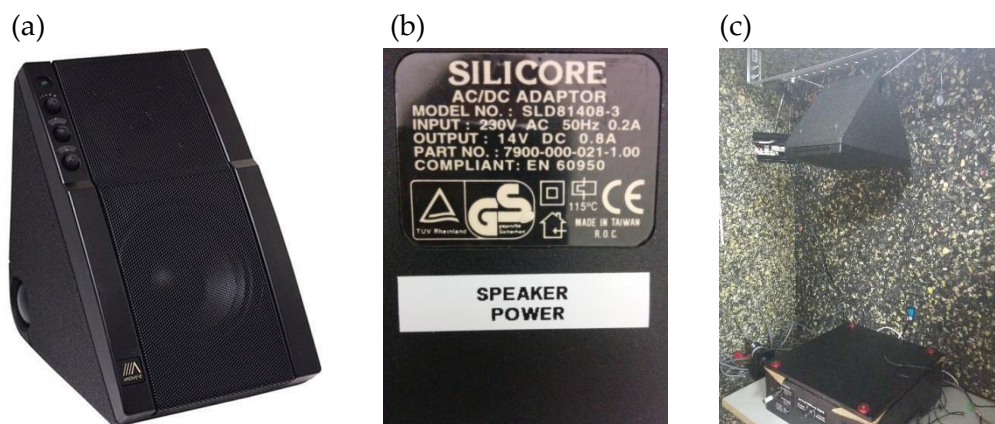


Figure 37 - (a) Advent AV570 speaker; (b) 14 V - 0.8 A power source; (c) speaker setup

Microphone

A microphone would allow the user to retrieve sound information from the interior of the chamber. A female jack terminal was welded to the inner terminal of the cable corresponding to the microphone's signal, in such a way that the device can be easily installed. A higher quality microphone –the ZM MIC1 by Zalman (figure 38b) - was

acquired and tested. The microphone has a sensitivity of $-40 \pm 3\text{dB}$ and more than 58dB of signal-to-noise ratio [29]. Additionally and in order to boost the signal amplitude a stereo microphone preamplifier by Monacor (figure 38c) was purchased.

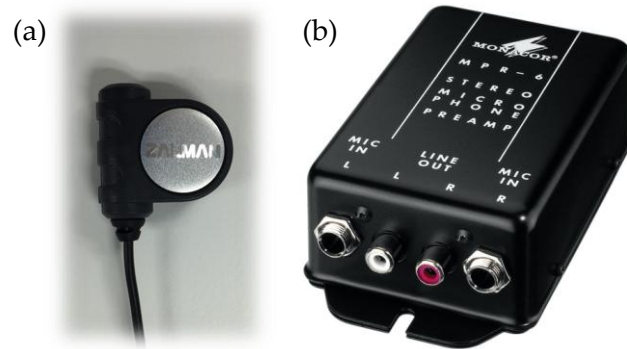


Figure 38 – (a) ZM-MIC1 microphone; (b) MPR-6 preamplifier

Connection to PC and sound archives

For audio digitalization we selected the USB digital audio I/O U-control UCA202 Behringer, Germany [30]. Through RCA ports it provides an audio interface that converts the analogue inputs into digital outputs. The device can be connected via USB port to the computer, which recognizes each individual port as a microphone or speaker. As shown in figure 39, the microphone was connected to one of the input ports and the speaker to one of the output ports. Taking the advantage of two ports being left open, the BCG signal was connected to an input port, hence allowing the device to perform the analogue to digital conversion of the signal. The device also includes a jack port that would allow the user to check whether the sound is being played without jeopardizing the system by having to open the door.

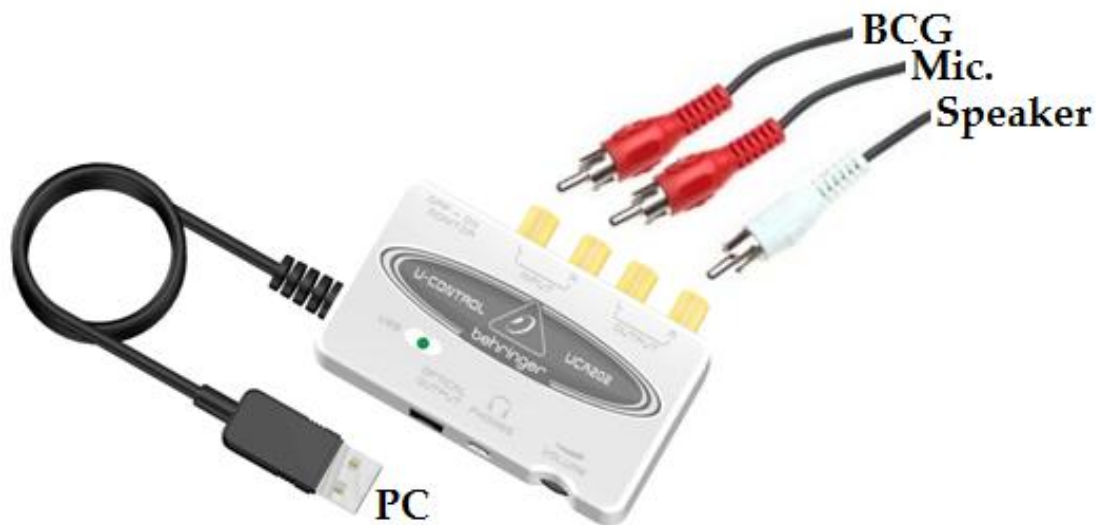


Figure 39 - Audio ADC and schematics of the connections to the audio devices and the BCG.

Regarding the previously mentioned sounds to be played in order to stimulate the chicken embryo, researchers developed two audio files for the purpose. The mother

call, which was intended to be the representative sound for the embryo, was recorded from a hen. The frequency spectrum of this recording was used to create a non-vocal timbre without any information for the chicken but maintaining similar temporal and spectral properties. This synthesized audio file represented the fake sound. Figure 40 compares the mother call to the fake sound and shows their spectral similarity.

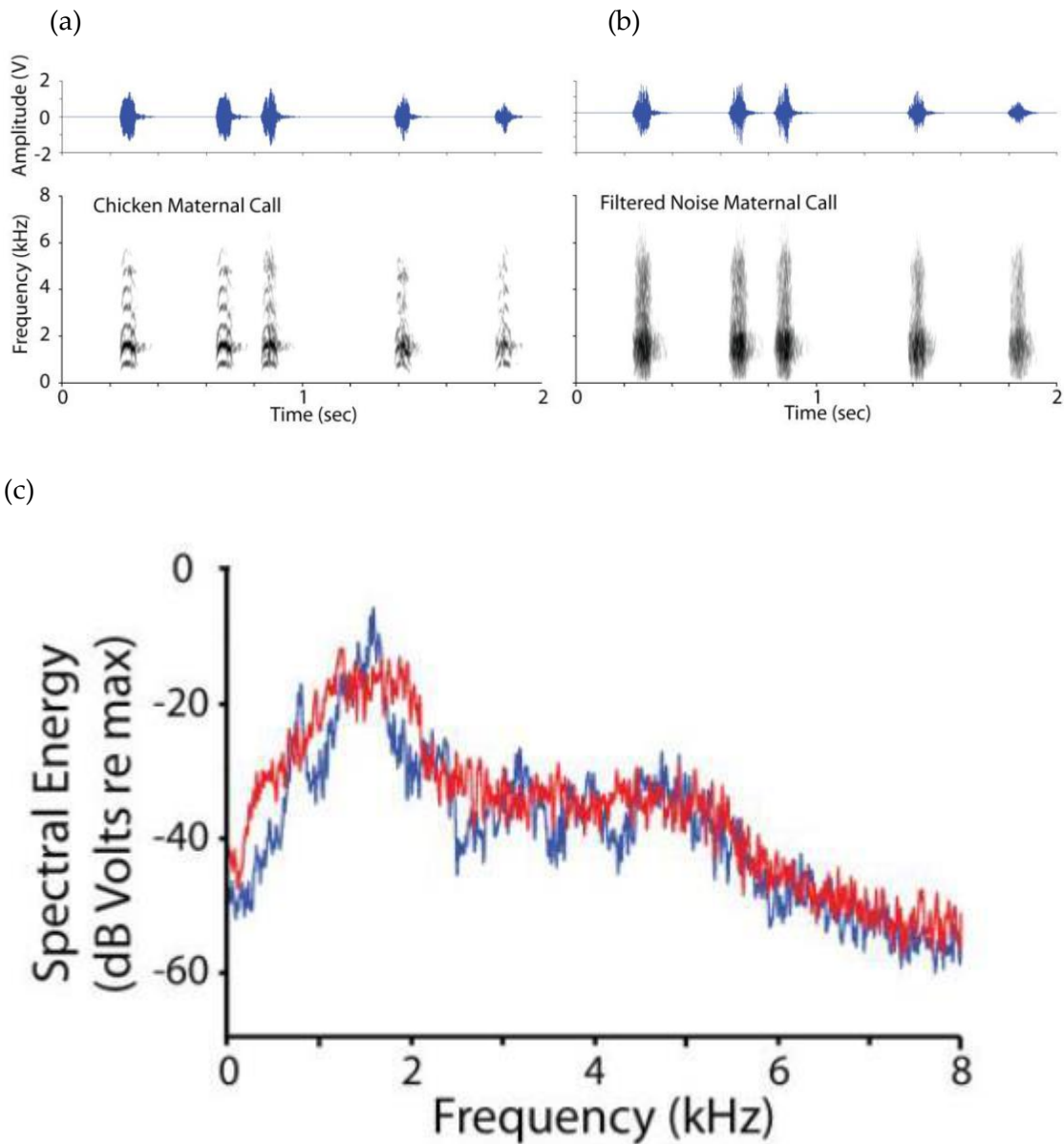


Figure 40 - (a) Mother call; (b) Fake call; (c) Comparison of spectral energies. Figures obtained from [8].

Information on how the user was able to select which sound to play, when to play it, whether to record with the microphone or not and whether to play the acquired sound is given in the User interface section of the chapter Results.

3.1.5 Lights and video recording

A system of lights and a webcam were installed inside the chamber in order to allow visualization of the cavity without the need of opening the door, hence minimizing human interaction with the inner environment.

Lights

Flexible strip led lights were acquired from PowerLED (figure 41a). The lights have ultra-low power consumption (2.4W per meter) and include a 12V driver that was mounted in the lower part of the chamber, together with the other power sources. The strip is 2m long and has 30 LEDs per meter, each of them with a 120° beam angle. The lights are warm white (3000K) and have a life of 30000 working hours [31].

The reasons behind selecting this product were its low power consumption, its long working life, its low price (refer to the *Costs* section for further information), and its easy installation system. The lights were installed inside the chamber by attaching them to a plastic surface that was previously secured to the walls of the chamber, as shown in figure 41b.

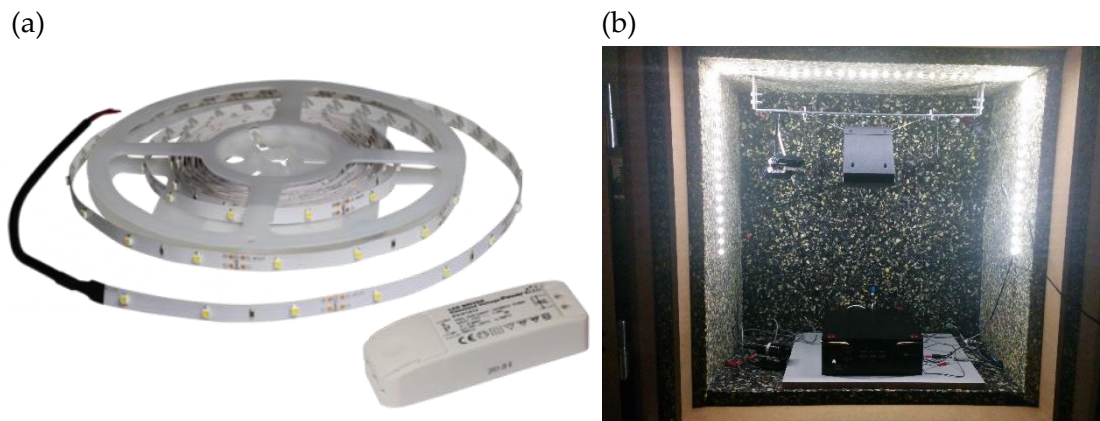


Figure 41 - (a) Warm white led; (b) Lights setup.

The user interface described in further sections of the text allowed the user to interact with the illumination system by deciding whether to turn the lights on or off by means of a relay controlled by a digital output pin in the Arduino board. When the user pressed the *turn on* button in the interface, LabVIEW communicated with Arduino, which set the digital output pin HIGH, hence closing the relay so that the lights were turned on. The opposite occurred when the user pressed the *turn off* button. The system is depicted in figure 42 and further information on how the user interface works is given in the *User interface* section of the chapter *Results*.

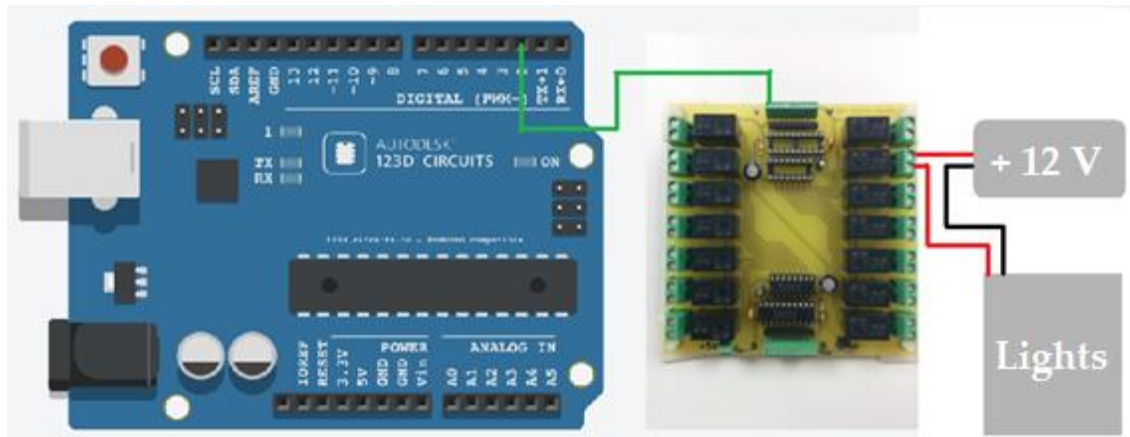


Figure 42 - Connections between Arduino, the relay, the power source and the system of lights.
(Schematics for illustrative purposes)

Webcam

A plug-and-play solution was found for the video acquisition system. The acquired device is an infrared webcam developed by CKB Ltd. It has small dimensions (13,2 x 8,4 x 3,8 cm), a 12 Megapixel resolution and nocturne vision [32]. It communicates with the computer via USB, which facilitates the installation and allows the product to be exchanged for a different one without further complications.



Figure 43 - Infrared webcam.

3.1.6 Microcontroller and relays

The selected board for communication of the system with the computer was Arduino [33], shown in picture 44. The board consists of an Atmel 8-bit AVR microcontroller [34], digital I/O pins, analogue pins and other components that facilitate programming and connection to other systems. The board comes pre-programmed and assembled. The code that controls its behavior is written in Arduino language and is then easily uploaded to the board through USB communication.

In particular, the Arduino UNO board was acquired and implemented in the system. The board provides 14 digital I/O pins and six analog inputs. Other features of the board include 32 KB of flash memory, 2 KB of SRAM and 1 KB of EEPROM. The size of 68.6 x 53.4 mm allowed the Arduino board to be kept inside the control box secured to the lateral of the box.

For the implementation of the system described in this text, two digital outputs and one analogue input of the board were used. In order to allow communication in real time between the board and the computer, the former was constantly connected to a USB port of the PC.

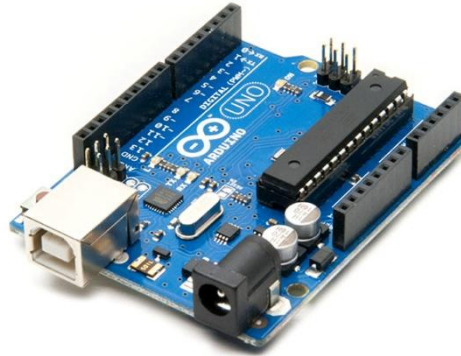


Figure 44 - Arduino UNO board

The digital outputs of the Arduino board were connected to respective pins of a system of relays. Each relay, which is an electrically operating switch, was then wired to the power supply of the heater and the LED lights. The board was powered with a +5V supply.

The board of relays (figure 45) was designed and developed upon request at the electronic technical office of Universidad Carlos III de Madrid. The schematic is included as an appendix at the end of the document. The board's dimensions allowed the system to be fitted inside the control box together with the Arduino board and other connections.

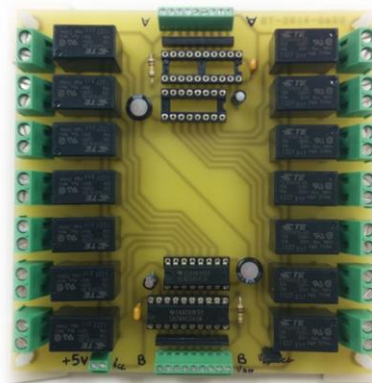


Figure 45 – Relays card

3.1.7 PC and user interface

A user-friendly interface that allows the user to interact with the equipment located inside the chamber has been developed in LabVIEW 2013 (National Instruments). The code is attached as an appendix of the document. The developed software displays

real-time temperature and humidity data with upper and lower warnings and allows the user to turn on and off the lights, to play real or fake calls to the developing embryo, to visualize the data recorded with the BCG, to record with a microphone what happens inside the chamber and to replay such a recorded sound. Figure 46 shows the overall appearance of the interface. Behind the LabVIEW interface, a code developed in Arduino communicates through the Arduino board with the inside of the chamber. In addition to the software developed in LabVIEW, the computer interface includes the display of the EEG and the webcam. The data retrieved by the pyrometer is displayed on an external display.

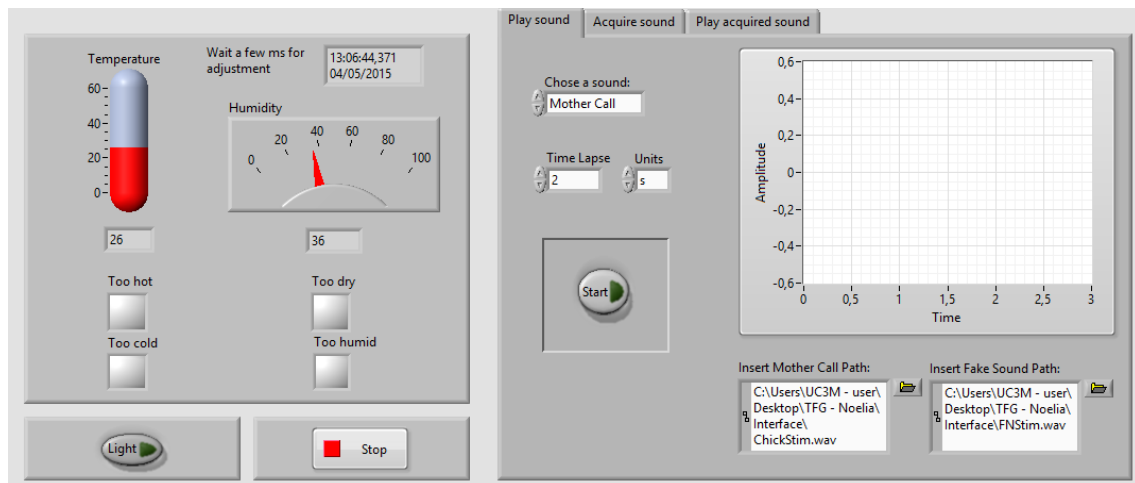


Figure 46 - Interface in LabVIEW.

Temperature and humidity

Temperature and humidity values are recorded with the aid of a sensor that communicates with the Arduino board. Through serial communication, the LabVIEW asks for temperature and humidity information to the Arduino board, which in turn sends it to be displayed on the interface. As shown in figure 47, temperature and humidity are shown to the user in a very intuitive way. When the temperature falls below 25°C or rises above 28°C, warning LEDs are turned on. In a similar way, a warning appears when humidity is below 30%RH or above 80%RH. Temperature and humidity are automatically controlled by the system, in such a way that the warnings show when the system failed to control the environment, requiring human immediate action. The way in which temperature and humidity are controlled is detailed in the section *Automatized temperature and humidity regulation*. The software creates temperature and humidity logs in excel files.

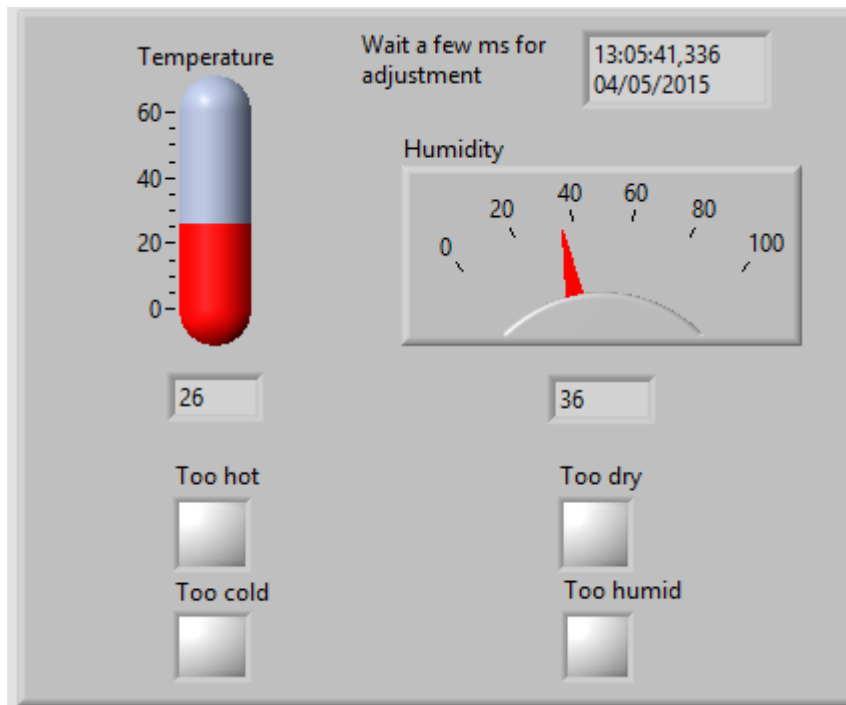


Figure 47 - Display of temperature and humidity values.

Lights and webcam

The interface allows the user to turn on and off the lights inside the chamber by clicking on the button “light”. When this button is pressed, the LabVIEW code communicates with Arduino through the serial port. When Arduino receives the command, a digital pin is set HIGH, meaning that a 5V voltage is sent to that particular pin, which is in turn connected to a relay. The relay is then closed and the lights receive power that enables them to turn on. Figure 48 shows the “light” button pressed and the inside of the chamber illuminated. The box interior is monitored by the webcam and the image is displayed with a its own software.

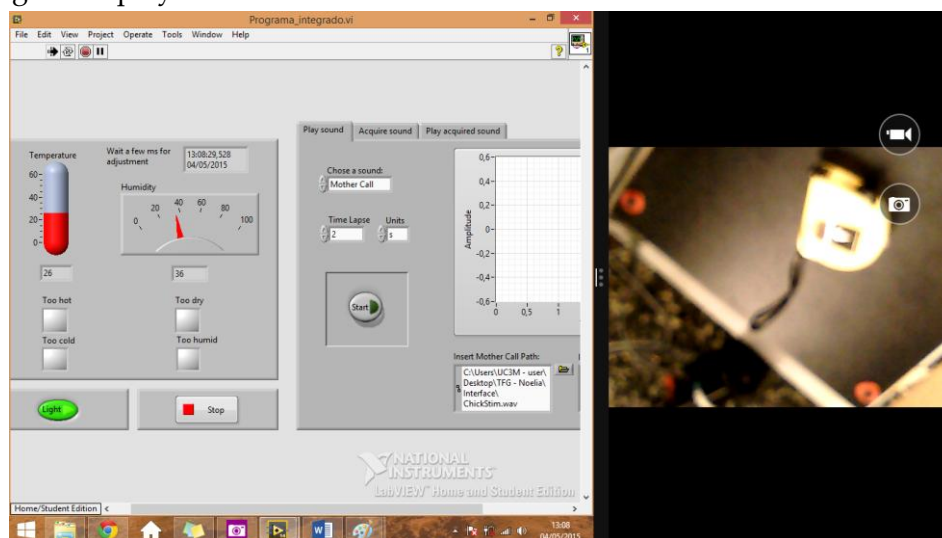


Figure 48 - Visualization of the inner chamber with the lights turned on by the user. The “light” button is green when the lights are on.

Play sound

As mentioned in previous sections of the text, it was of particular interest for research purposes to study the response of the chicken embryo to acoustic stimulation. Therefore, the interface allows the user to select whether to play the *Mother Call* or the *Fake Call*, as well as the time to wait between consecutive plays. When the user presses “start”, the speaker plays the selected sound inside the chamber. Figure 49 shows the appearance of the *Play sound* tab while the mother call is being played.

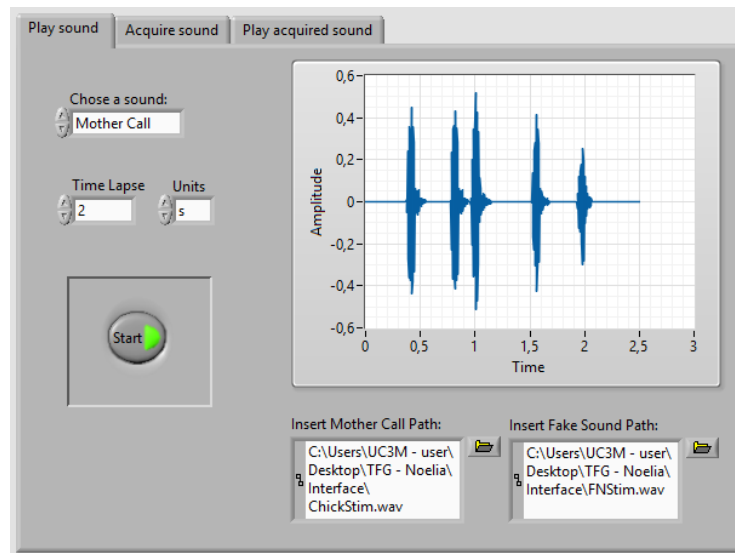


Figure 49 - Play sound.

Acquire sound

In a similar way, sound can be recorded from the inside of the chamber with the aid of the installed microphone. This tab also allows the user to record the signal detected by the BCG device. The sample rate, the number of channels and the bits per channel are parameters that can be easily modified by the user. Figure 50 shows the appearance of the *Acquire sound* tab once the *Acquire* button has been pressed. The audio file is saved in the computer for further analysis and processing.

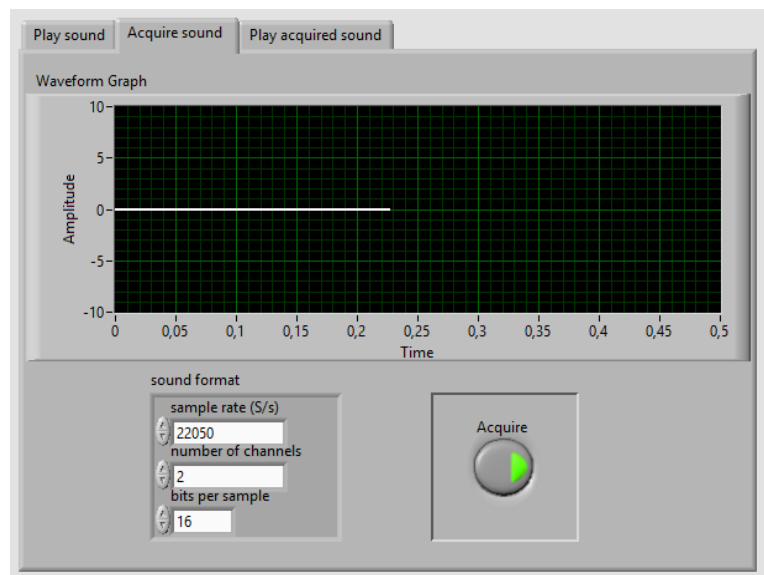


Figure 50 - Acquire sound. Only one channel is shown because at the moment of acquisition the BCG was not operative.

Play acquired sound

The last tab allows the user to replay the sound that was previously acquired. When the *Play* button is pressed, the sound will be played and the waveform will be represented on the screen.

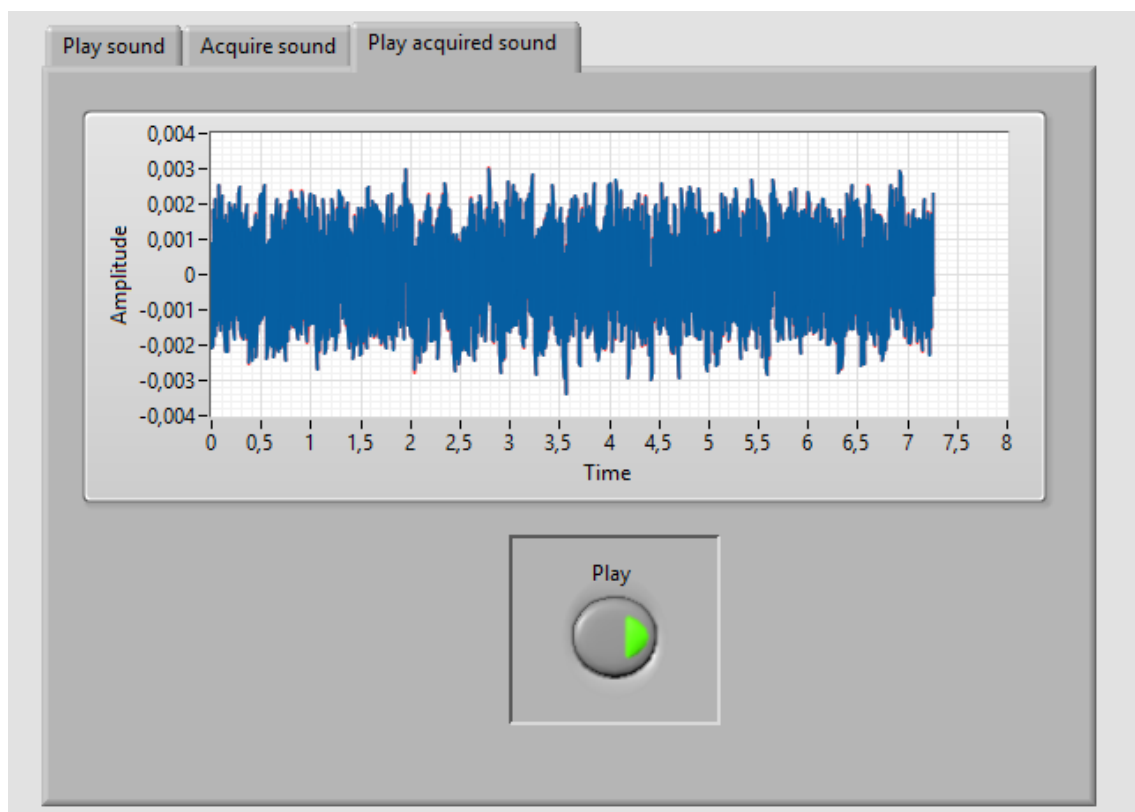


Figure 51 - Play acquired sound.

Figure 52 shows how other buttons are disabled while the *Start*, *Acquire* or *Play* buttons are pressed. This avoids conflicts between different commands.

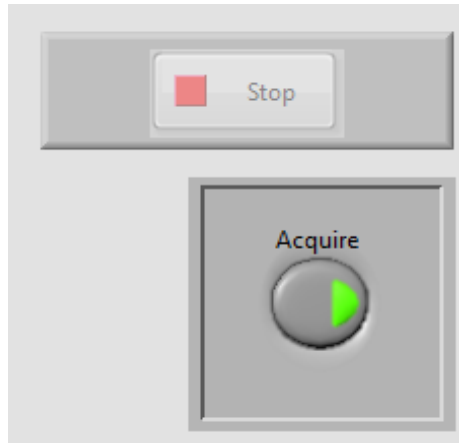


Figure 52 - When the *Start*, *Acquire* or *Play* buttons are pressed, all other buttons are disabled.

EEG display

The EEG systems developed by the Italian team works with a software that automatically launches when the EEG USB is connected to the computer. For the case of the other EEG system– the data would be also acquired with its proprietary software.

Pyrometer

Temperature data retrieved by the pyrometer is displayed in real time on its own display screen, which was mounted on the side of the chamber, as shown in figure 53.

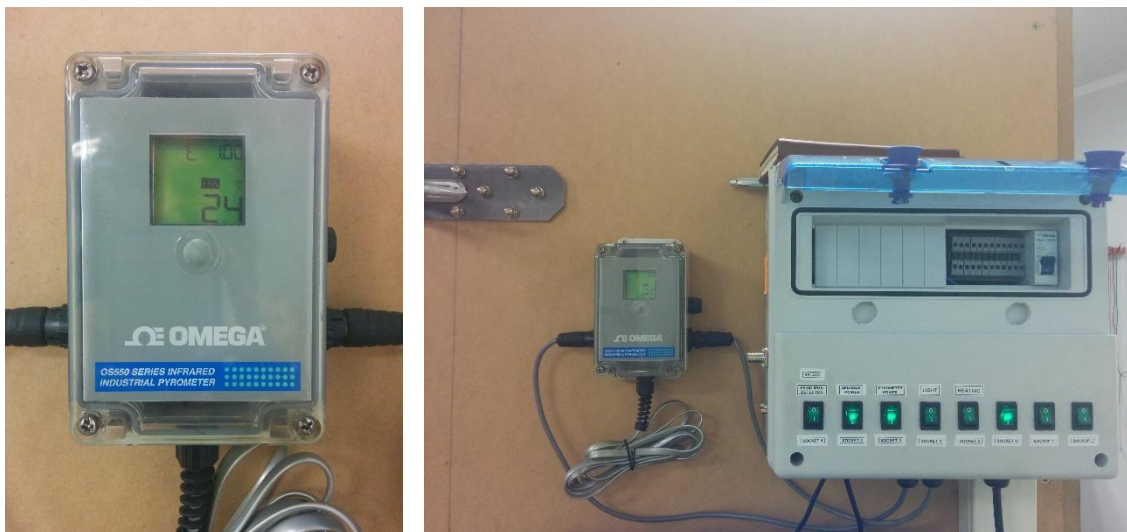


Figure 53 - Pyrometer's (a) display and (b) display's setup.

4 Conclusion

The main goal of the presented Bachelor Thesis was to develop a system for the recording vital signs from a chicken embryo *in ovo*, places inside and environment-controlled chamber. The general design of the system that was done in a previous TFG, and in this project the systems was built integrating the different components that have been described in this text.

The system is ready to be used in order to conduct animal trials for the study of embryonic brain development. The system will also allow for future biomedical engineering students to design and implement new sensors and test their capabilities.

4.1 Current status of the system

The system is fully built and the technical and user requirements have been satisfied. The isolation chamber is constructed and it complies with the space conditions as well as with the constraint of the acoustic and electrical isolation. The system has been wired in order to allow for easy connection of the EEG and BCG for vital signs monitoring (plug and play).

Air and temperature humidity are recorded and displayed in real time and the inner environment is automatically regulated in order to minimize human intervention with the cavity.

A speaker has been installed so as to allow the researches to acoustically stimulate the growing embryo, and a user friendly interface has been developed to offer them an easy way to select the desired sound to be played.

In order to further reduce human interaction with the inner environment, lights, an IR webcam and a microphone have been installed inside the chamber. With the aid of the aforementioned user interface, the personnel can monitor the inner cavity without the need of opening the chamber's door.

Communication with the PC, the Arduino microcontroller, a system of relays and the sensors and actuators have been unified in a control unit that also displays all the parameters of the chamber environment.

4.2 Next steps

4.2.3 Future guidelines

Future work on the system will be related to the EEG components: a new USB version is coming and there are cables ready for installation, but nevertheless, it will have to be tested.

The chamber is ready to be used by researchers that wish to study brain development on embryonic avian models. Animal trials can start once the new BCG sensor is connected.

4.2.3 Future improvements to the system

The system was designed in a modular way that envisioned the possibility of further improvements of the system without disturbing the overall research procedure. The nature of the design was not modified, and the final result is a sum of individual components that work in conjunction with each other while offering the possibility of being replaced by new modules that can be easily integrated in the system.

Each module of the system can be replaced by devices that offer characteristics that fit better with the nature of the research being carried out at each moment. Not only the sensors and actuators can be replaced but also the power sources and the user interface.

It is intended that each user decides what works better for their requirements and specifications. This intention is clearly expressed in the presentation of two different EEG acquisition systems that can be integrated according to the researcher's needs.

Other EEG options are the sensors developed by Pedro Irazoqui's team at Purdue University [35] and biocompatible electrodes made with organic electrochemical transistors [36].

The system offers many possibilities for brain researchers and for sensors developers to explore new areas of research or to test new ideas. Researches are encouraged to use the system as well as to ask for more modules to sense and interact with the inner environment that hosts the embryo. An empty cable-bundle track has been left empty in order to facilitate the installment of new devices without disrupting the electrical isolation of the chamber.

Engineers are encouraged to develop new sensors that satisfy the needs that future researchers will have, as well as to improve those sensors that are currently installed in the system. A line of research can be oriented towards the betterment of the BCG device. Some suggestions include the replacement of the measuring method by laser vibrometry [37] or the replacement of the phonograph needles by IMUs (Inertial Measurement Units) [38].

Other components to be modified are the temperature and humidity sensor –if higher accuracy is required-, the webcam –if higher resolution is desired- and the pyrometer – a small unit will be more convenient-.

5 Costs

Budget of the project			
1.- Author			
Noelia Lombardo Gava			
2.-Department			
Biomedical and Aerospace Engineering			
3.- Project description			
- Title	Instrumentation of an isolation chamber for the study of brain development in avian models		
- Duration (months)	9		
Indirect costs:	20%		
4.- Project budget (€)			
48.532,11			
5.- Budget breakdown			
Human resources			
Category	Human-months	Cost of human month	Cost (€)
Technical Engineer	1	2.280,00	2.280,00
Engineer	8	2.694,39	21.555,12
		Total	23.835,12
Materials			
Description	Cost (€)		
Antivibration table	1.600,00		
Arduino	25,00		
Audio A/D	170,00		
BCG	500,00		
Chamber	2.500,00		
Computer	900,00		
EEG	2.000,00		
Heater	7,68		
Light	36,58		
Microphone	15,00		
Pyrometer	320,00		
Reles	65,00		
Speaker	260,00		
T&H sensor	10,00		
Webcam	40,00		
Total	8.449,26		
Details of material cost for BCG development			
RS code	Description	Quantity	Price (€)
487832	Conec Hembra Aliment Panel 2.1mm 5A 12V	1	3,59
572407	FC radial elec cap 1800µF 10V	10	6,00
1939072	Connector HF Coax SMA J01151A0021	2	12,46
5173377	Natural aluminium box, 80x55x25mm	1	4,95
5463361	Conector hembra recto de crimpado SMB	4	15,96
6563816	Dcpower adaptor straight plug lead 2.1 mm	2	4,10
6604475	Comparator single R-R I/O 5.5V 6-pin	1	2,15
6992817	Capacitor tantalum TAJ 1206 2.2µF 10V	10	6,03
7884151	SMD PVG3A Trimmer single turn 20R 0,25W	1	0,83
7900493	Capacitor snap-in UP series 68µF 400V	1	2,46
8122741	Aluminium radial capacitor 680µF 25Vdc	10	4,55
		Total	63.08

Details of material cost for chamber construction					
Description	Price (€)	IVA (€)	Total (€)		
Aluminum profile 45x45	164,10	34,46	198,56		
Aluminum square 86x86x43	55,60	11,68	67,28		
Bearing	20,00	4,20	24,20		
Beech slats	304,80	64,01	368,81		
Cut the foam	95,00	19,95	114,95		
Hinge 20x150x4	11,58	2,43	14,01		
MDF 16	66,65	14,00	80,65		
MDF 19	98,55	20,70	119,25		
Plywood of 4	8,26	1,73	9,99		
Screw 8.8 M8x25 + screw M8	17,50	3,68	21,18		
Screw M12	18,60	3,91	22,51		
Wheel with break M12x70	37,52	7,88	45,40		
Wheel with break M12x70	19,46	4,09	23,55		
Wheel without break M12x70	25,72	5,40	31,12		
Wheel without break M12x70	12,02	2,52	14,54		
White glue 500g	3,86	0,81	4,67		
		Total	1.160,66		
Other direct costs					
Description	Company	Cost			
Internet	Telefónica	960,00			
Windows 8	Microsoft	99,95			
Office 2013	Microsoft	79,99			
	Total	1.139,94			
6.- General costs and industrial benefit					
On direct human resources costs	3813,6192	16%			
7.- Summary of costs					
Description	Budget on total costs (€)				
Personnel	23.835,12				
Materials	8.449,26				
Functioning costs	1.139,94				
Indirect costs	6.684,86				
Total without IVA	40.109,18				
IVA 21%	8.422,93				
Estimated total	48.532,11				

6 Appendices

6.1 User specifications

Report on State of User Requirements Specification

October – 2013

Proposal for User Requirements Specification

DATE: 10/10/2013

AUTHOR: CARMEN FERNÁNDEZ FISAC

EDITOR:

date	Author(s)	modifications
10/10/2013	Carmen Fernández Fisac	Initial Evaluation

Contents

INTRODUCTION.....	1
PRIORITY USER REQUIREMENTS	1
SECONDARY USER REQUIREMENTS.....	2
SYSTEM DIAGRAM.....	3

Introduction

The goal of the system is to non-invasively acquire, process and analyse fowl embryo EEG data, integrating it with other vital signs such as temperature, ECG and movement as well as other influential variables, which include environment conditions and acoustic stimuli, and allowing for further study and interpretation.

Priority User Requirements

Chamber

The chamber will ensure acoustic and electromagnetic isolation.

The chamber will have the capacity to accommodate the necessary sensors and controls in addition to the egg.

Log

The system memory will allow saving the data into a log of at least 180 hours.

The following variables will be registered in the log:

- Acquired Data:
 - o EEG signal
 - o ECG curve
 - o Sound inside the chamber
 - o Embryo temperature
 - o Chamber air humidity
 - o Chamber air temperature
 - o Video recording (always available, but not necessarily on)

Page 1 of 3

Report on State of User Requirements Specification

October – 2013

- System State:
 - o State of chamber illumination (on/off)
 - o State of video camera (on/off)
 - o State of speaker (on/off)
 - o State of chamber door (open/closed)

Controls

Automatic:

- The system will keep chamber air humidity in the range RH 30-80%, response time < 10 minutes.
- The system will keep chamber air temperature in the range 25°C - 38°C, response time < 3 minutes.
- The system will keep embryo temperature in the range ____*, response time < ____* minutes.

User Controlled:

- The operator will be able to turn on and off:
 - o the chamber lighting (produce stimuli)
 - o the video camera
 - o the speaker (produce stimuli and avoid unnecessary noise)
- The operator will be able to manually open and close the chamber door.

Display

The program will run on a standard WXGA monitor.

The user interface will exhibit the following sections:

- Log presenting all recorded parameters along time
- Graph featuring real time plots
- Recorded video
- Study conditions (session ID, aim, conductor, relevant egg demographic data)
- Date and time
- Running time
- Alarm

- When exiting acceptable chamber air temperature range
- When exiting acceptable chamber air humidity range
- When exiting acceptable embryo temperature range
- When detecting abnormal ECG activity
- Notification
 - When illumination is on
 - When video camera is on
 - When speaker is on
 - When door is open

Secondary User Requirements

The system will include 2 configurations:

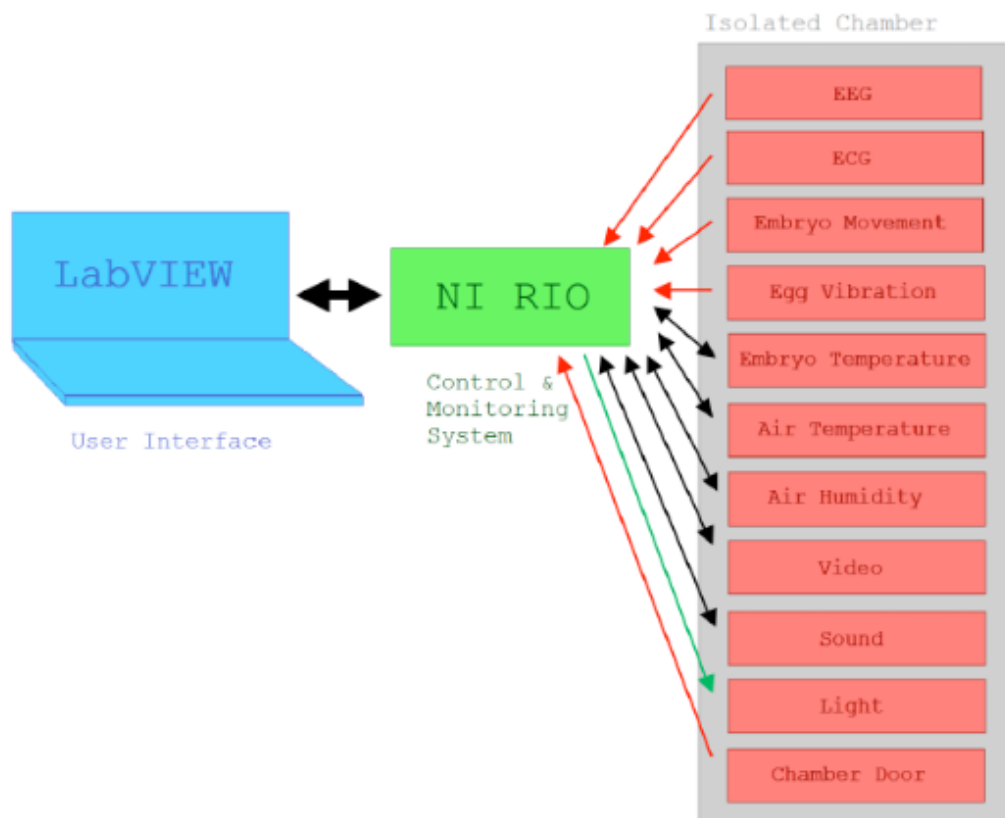
- Acquire new data
- Open existing file and replay a previous study from the console

The system will allow the user to leave notes in specific time stamps.

The video window will be hidden when it is not recording.

Page 2 of 3

System Diagram



* pending from *Università di Pisa*

Page 3 of 3

6.2 Technical specifications

Technical Requirements

Chamber

The chamber will hold:

- An egg
- Audio devices
 - o microphone
 - o loudspeaker
- Vibration isolation platform
- Balistocardiograph (BCG)
- Video camera
- Illumination
- Air temperature & humidity sensors & actuators
- EEG & embryo temperature equipment

Isolation Properties:

- Acoustic
 - o 90dB noise attenuation (at least 70dB, *15dB maximum ventilation noise*)
 - o Audible range for chickens: 30Hz to 10kHz
- Electromagnetic
 - o 90dB attenuation
 - o Eliminate 50 cycle noise (interferes with EEG reading)

Other Components:

- Door
 - o Dimensions: 700mm W x 700mm H
 - o Opened and closed manually from the outside
 - o Interlock (trigger alarm in LABVIEW when open)
- Handles
 - o 4 → held comfortably by 2 people
 - o 2 → allow single person transport
- Connection panel
- Air ventilation (in & out)
 - o Light trapping
 - o Sound trapping

Dimensions:

- Inside: no less than 800mm W x 600mm D x 800mm H
- Outside: no more than 1000mm W x 800mm D x 1000mm H

Monitoring Devices

Vibration Isolation Platform

- Dimensions: 310mm W x 310mm D x 117mm H
- Payload range: 0-4.5kg

Temperature & Humidity Sensors

- Analogue Temperature Sensor with Signal Conditioning
 - o Unit AD22100ATZ
 - o Temperature range: -40 – 85 °C
 - o Output range: 0.475-3.288V (22.5mV/°C)
 - o $V_{out} = V_{supply}/5V * (1.375V + 22.5mV/°C * T_{air})$
 - o Accuracy: $\pm 0.5^\circ C$
 - o Response time $\leq 2s$
 - o Power supply: 5V
 - ✓ Additional Industrial IR Pyrometer
- Analogue Low Voltage Humidity Sensor
 - o Unit HU5031 (Honeywell Sensing)
 - o Output range: 1V-3V
 - o Accuracy: $\leq \pm 5\%RH$ at 25°C, 25 ~ 90%RH
 - o $V_{out} (V) = V_{supply} * (0.00636 * \%RH + 0.1515)$
 - o T compensation: True RH = $\%RH / (1.0546 - 0.00216 * T_c)$
 - o Response time $\leq 5s$
 - o Power supply: 3.3Vdc

Balisticardiograph: “Huevomovimetro”

- Current system:
 - o Eggs stand on 3 needles that detect its vibration
- Other possible models:
 - o Laser vibrometry
 - IR laser
 - Reflection sensor captures light from egg surface
 - o Accelerometers/gyroscopes
 - Invensense MPU 6500
 - Accelerometer + gyroscope = 6 axis
 - Place on highest movement spots
 - SPI interface
 - Power supply: 1.8-3.3V
 - Dimensions: 3mmx3mmx0.9mm (NO radiotransparency available in the market)
- Separate BCG signal from embryo movement and egg vibration

EEG & Embryo Temperature

- Output via RS-232
- Data stored in *.txt file
- Alternative embryo temperature:
 - o Industrial Infrared Thermometer (IIR)
 - Analogue output: 0-5Vdc
 - Egg surface emissivity 1.00 (calibrated 2008)

Video Camera

- Focus in the cm scale
- Resolution around 640 x 480 pixels, 30fps
- Output format
- Possibly integrated microphone (unknown specifications)
- IR LEDs ring

Microphone

- Requirements:
 - o Small
 - o Frequency range around 100Hz to 15kHz
 - o Omnidirectional
 - o Good sound quality
- Possible parts:
 - o Instrumental Microphone (for drum suite)
 - Unit Hi-10
 - Sensitivity: -38dB (12.6mV/Pa)
 - Frequency range: 50-18k (Hz)
 - Power supply: 9-48 Phantom
 - o Omnidirectional Microphone
 - Unit ADMP401
 - Bottom port
 - Sensitivity: -42dB
 - Frequency range: 100-15k (Hz)
 - Power supply: 1.5-3.3 V

Control Devices**Heater**

- Account for both air & embryo temperature
- Response time < 3 min
- Not supply too much heat that would endanger the chicken
- Easily fabricated but could also be ordered

Humidifier

- Response time < 10 min
- Easily fabricated but could also be ordered
- Blotting paper and perfusion pump
- Automatic control through software

Air Ventilation

- SILENT
- a) Fan(s) constantly ON to eliminate the vertical temperature gradient
- b) Air vent (in & out) to change the air in the chamber
- c) Automatically controlled ventilator
 - o Account for excessive temperature and humidity
 - o Response time < 3 min

Chamber Illumination

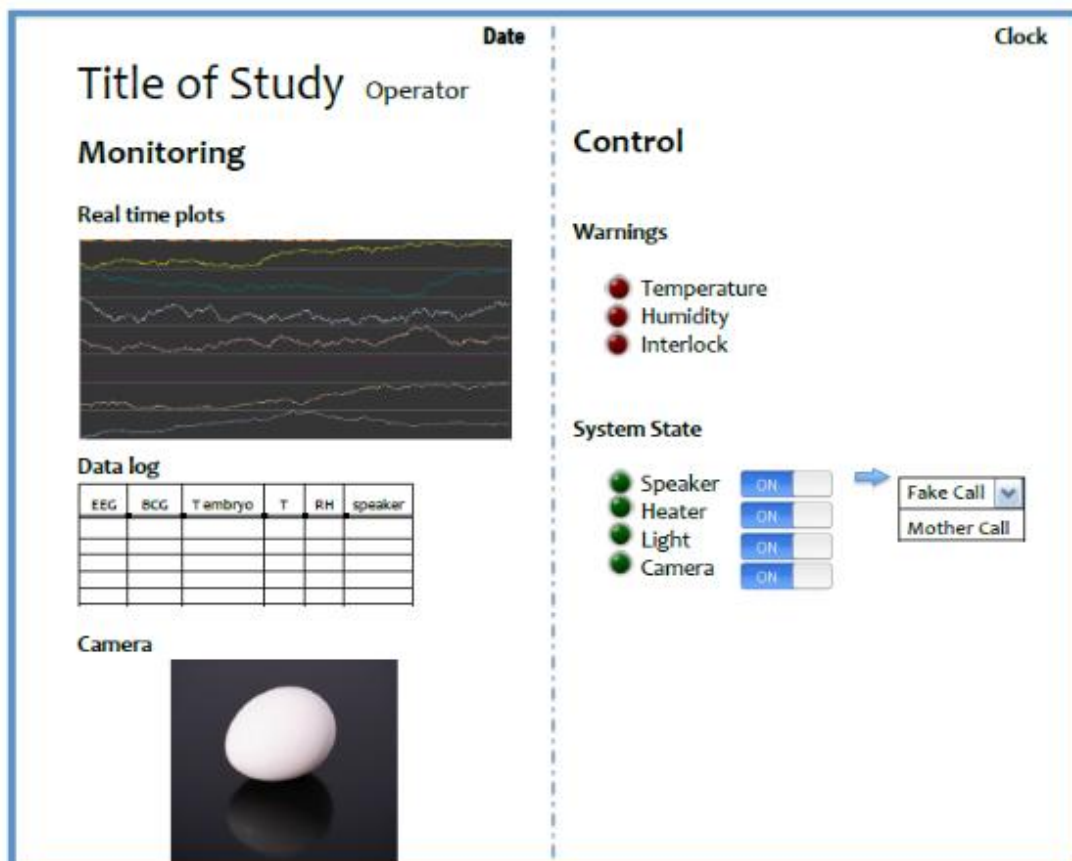
- LEDs stripe containing RGB components
 - o White light for chamber inside manipulation
 - o Low red wavelength to avoid disturbing/stimulating chicken
- Intensity

Speaker

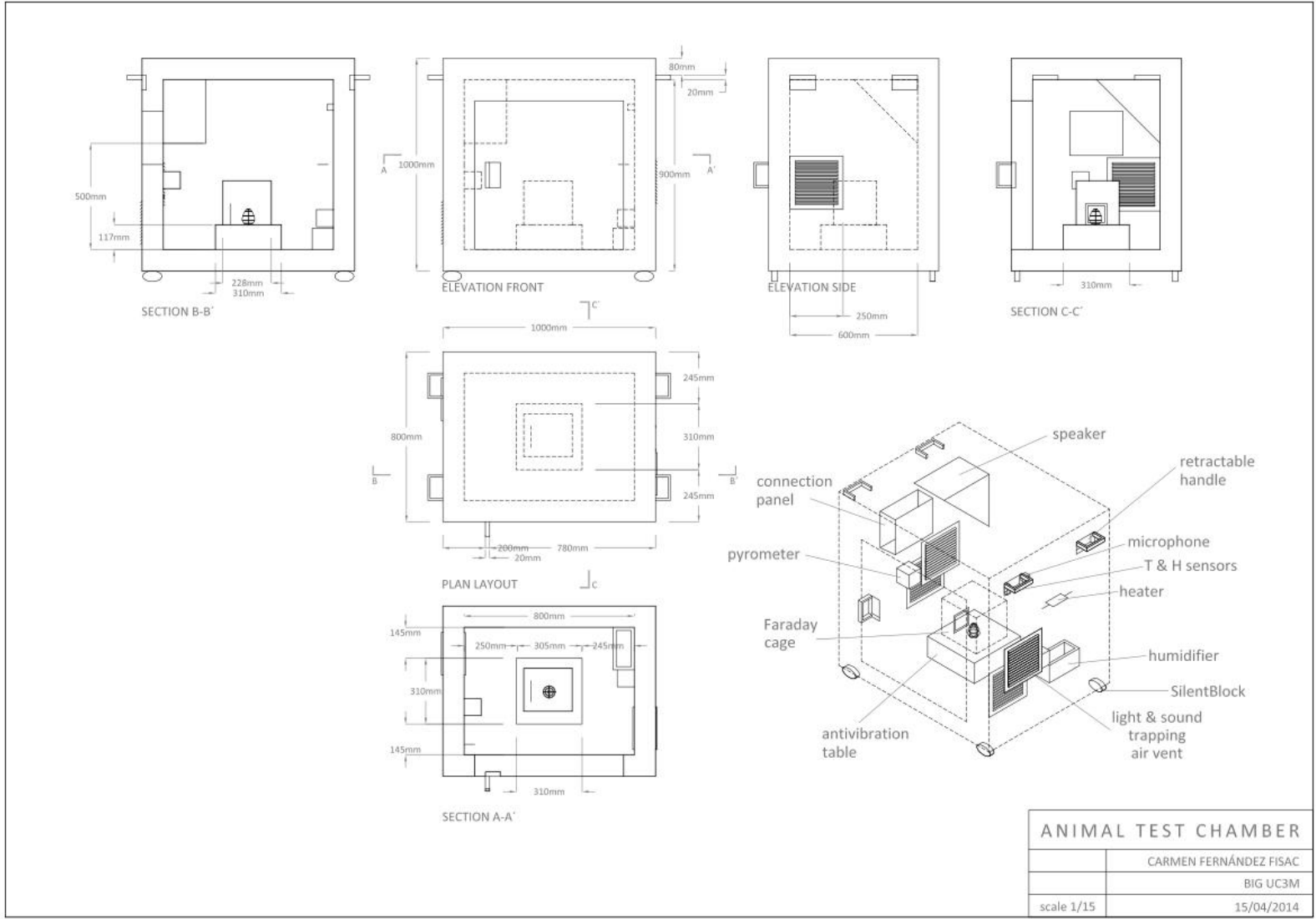
- Single speaker playing right and left channels
- At least 90dB pressure: 5W acoustic power would suffice
- Current speaker:
 - o Power supply: 14Vdc (0.8A has proven to be enough)
 - o Acoustic power: 70W (overkill)

Display**LabVIEW User Interface**

- Monitoring
- Control



6.3 Chamber plans



The chamber has final dimensions specified on page 12 of the document.

6.4 Arduino code

```
#include <DHT.h>

#define dhtpin A0 //where sensor is connected
#define dhttype DHT11 //change if sensor is replaced by other model
#define pinLight 7
#define pinHeater 8

DHT dht(dhtpin,dhttype);

char in;
int temp;
int hum;

void setup() {
  Serial.begin(9600); //baud rate 9600, begin serial communication
  dht.begin(); //begin sensor communication
  pinMode(pinLight, OUTPUT);
  pinMode(pinHeater, OUTPUT);
}

void loop() {

  if(Serial.available() > 0)
  {
    in = (byte)Serial.read() & ~(0x20);

    //The heater will be turned on when the temperature goes below
    //26 and off when the temperature is above 28
    temp = dht.readTemperature();
    if (temp < 26)
    {
      digitalWrite(pinHeater, HIGH);
    }else if (temp > 28){
      digitalWrite(pinHeater, LOW);
    }

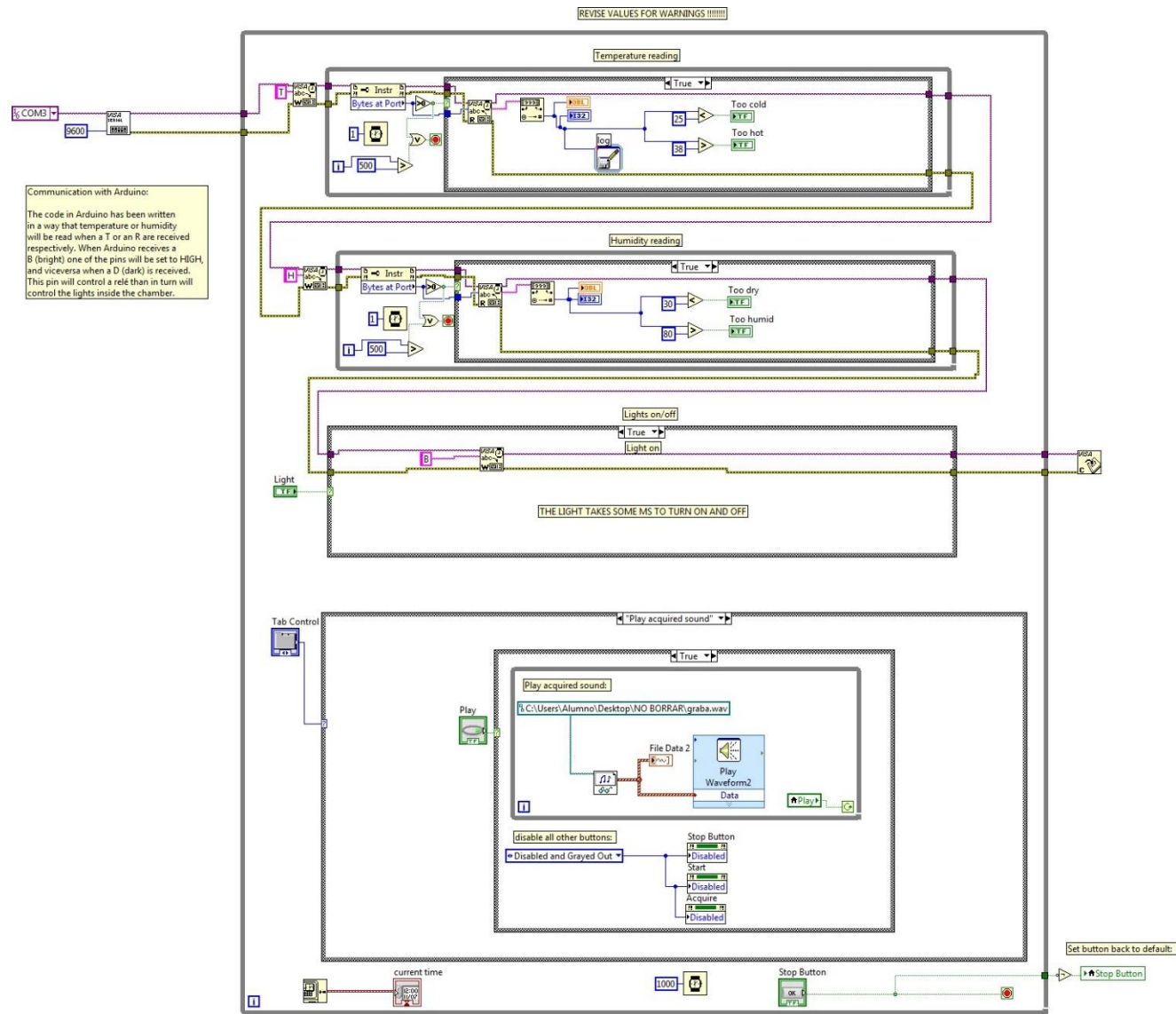
    if (in == 'T') // Temperature
    {
      temp = dht.readTemperature();

      Serial.println(temp);

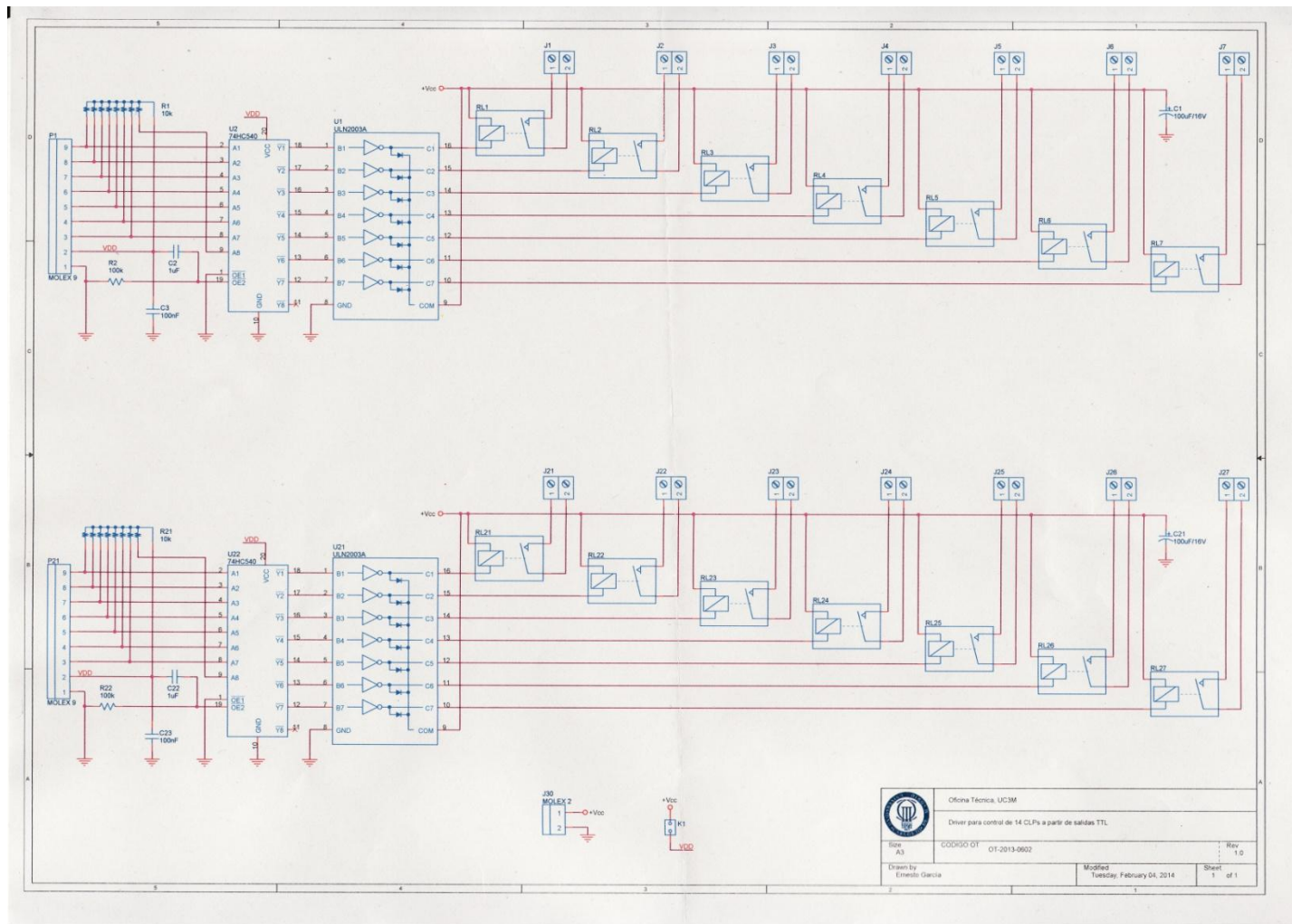
    }else if (in == 'H'){
      hum = dht.readHumidity();
      Serial.println(hum);

    }else if (in == 'B') //LIGHT ON (bright)
    {
      digitalWrite(pinLight, HIGH);
    }else if (in == 'D') //LIGHT OFF (dark)
    {digitalWrite(pinLight, LOW);}
  }
}
```

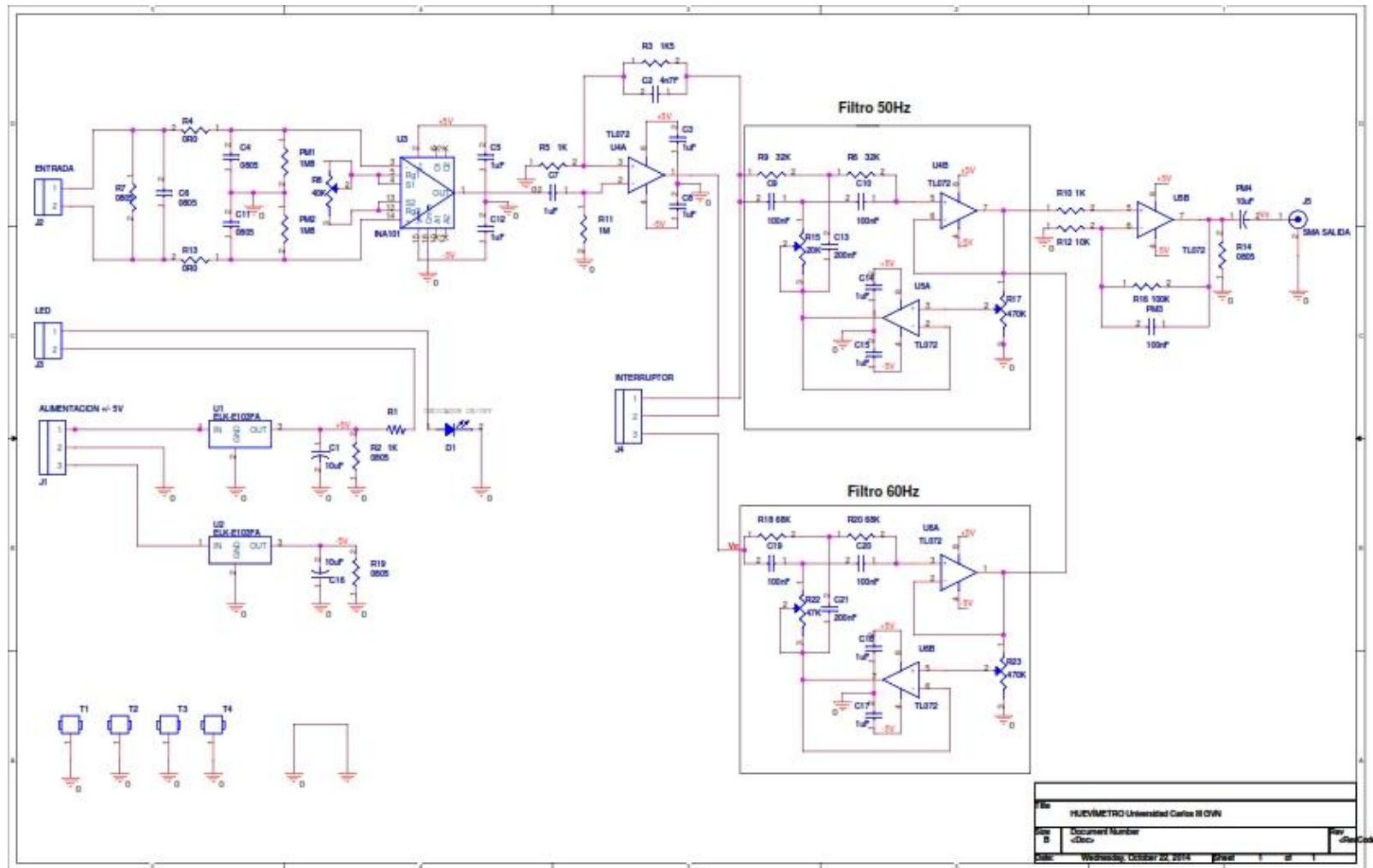
6.5 LabVIEW code

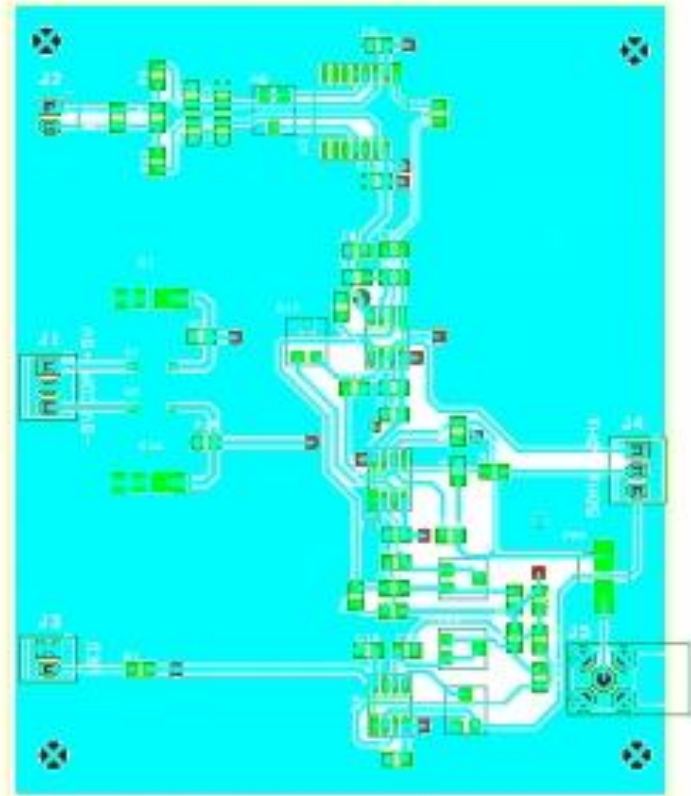


6.6 Relays schematics



6.7 BCG schematics





DRILL CHART				
SYM	DIAM	TOL	QTY	NOTE
■	0.700 mm		17	
+	0.711 mm		1	
x	1.016 mm		10	
◇	1.600 mm		5	
+	2.997 mm		4	
TOTAL			37	

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